

Flexible synthetische Zugänge zu bioaktiven O-
und N-glykosylierten 3-Acyltetramsäuren aus
Meeresschwämmen –
Erstsynthese von Aurantosid G, Ancorinosid A
und Methyl-Ancorinosid D

DISSERTATION

zur Erlangung des akademischen Grades eines
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Meiner Familie

“The good thing about science is that it's true whether or not you believe in it”

Neil DeGrasse Tyson

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Zusammenfassung

Ziel dieser Dissertation war die vollsynthetische Darstellung natürlicher glykosylierter 3-Acyltetramsäuren. Tetramsäuren und insbesondere ihre an C-3 acylierten Derivate stellen ein in der Natur häufig vertretenes Strukturmotiv dar. Eine Vielzahl von Naturstoffen mit diesem Motiv weisen interessante biologische Wirkungen wie antibiotische, antivirale oder auch zytotoxische Effekte auf. In Folge dessen sind 3-Acyltetramsäuren, insbesondere im Laufe der letzten Jahrzehnte, zu einem häufig untersuchten Ziel geworden und auch für Synthetiker in den Fokus gerückt. Obwohl bereits eine Vielzahl an natürlichen 3-Acyltetramsäuren untersucht und synthetisch dargestellt wurde, gibt es auf Grund der strukturellen Vielfalt keine allgemeingültige Strategie für ihre Darstellung. Besonders in der Gruppe der glykosylierten 3-Acyltetramsäuren sind nur wenige Derivate synthetisch in Angriff genommen und deren Wirkungen im Detail erforscht worden. Ausgangspunkt dieser Arbeit war deshalb, noch nicht synthetisch untersuchte *O*- und *N*-glykosylierte 3-Acyltetramsäuren totalsynthetisch darzustellen. Ein zusätzliches Ziel war, einen möglichst modularen Zugang zu den einzelnen Klassen zu schaffen. Hierdurch wäre es möglich Derivatisierungen an verschiedenen Stellen durchzuführen, beziehungsweise nicht nur einzelne ausgewählte Naturstoffe darzustellen.

Im ersten Abschnitt dieser Dissertation wurde die Synthese von *N*-glykosylierten 3-Acyltetramsäuren bearbeitet. Hier sind neben dem literaturbekannten Streptolydigin (**20**) bis heute nur die Vertreter der Rubroside (**74**) und der Aurantioide als natürliche 3-Acyltetramsäuren bekannt. In der vorliegenden Arbeit war es möglich Aurantioid G (**73**) als ersten Vertreter der konjugiert ungesättigten *N*-glykosylierten 3-Acyltetramsäuren totalsynthetisch darzustellen.

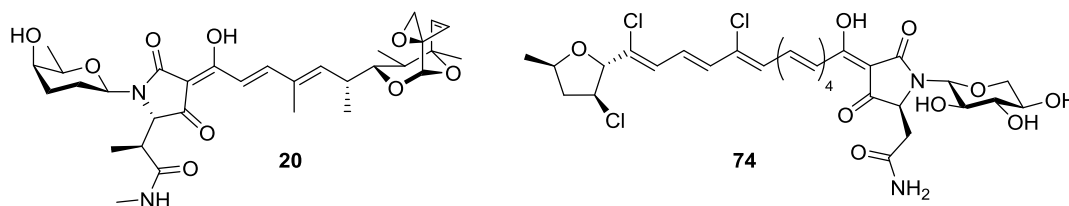
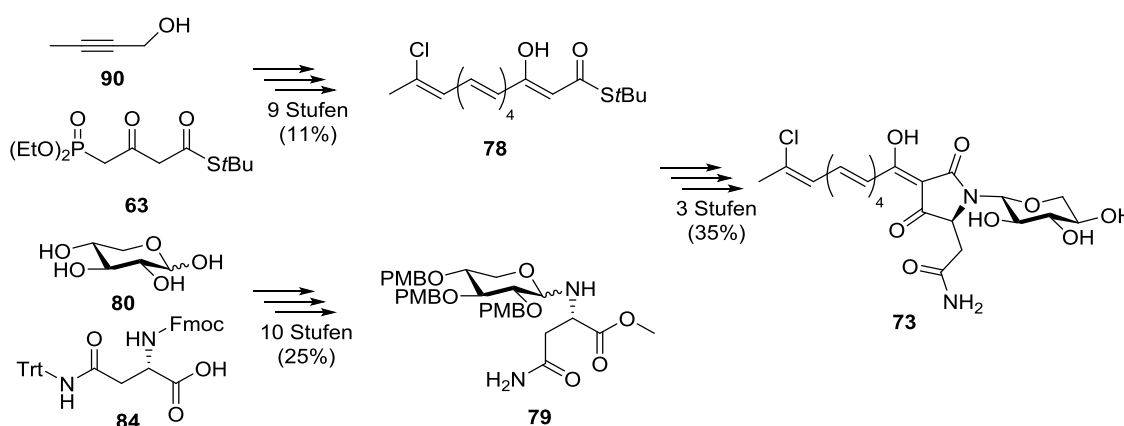


Abbildung 1: Struktur der *N*-glykosylierten 3-Acyltetramsäuren Streptolydigin (**20**) und Rubrosid H (**74**).

73 wurde aus zwei Teilfragmenten, der halogenierten vollständig konjugierten Seitenkette **78** und dem *N*-glykosylierten L-Asparaginat **79** aufgebaut. Die Seitenkette wurde dabei über neun Stufen mit einer Gesamtausbeute von 11% aus dem kommerziellen C-4 Baustein **90** mit schrittweiser Tandem-Oxidations-Wittig Reaktionen aufgebaut. Das *N*-Glykosid **79** wurde mit insgesamt 25% Ausbeute aus natürlicher D-Xylose (**80**) und einem ebenfalls erhältlichen, geschützten L-Asparagin **84** über zehn Stufen erhalten. Die Kondensation der Teilfragmente in einer silbervermittelten Aminolyse lieferte ausschließlich das β -Epimer. Eine abschließende Entschützung mit darauffolgender Lacey-Dieckmann Zyklisierung ermöglichte die Darstellung von **73** in drei weiteren Stufen mit 35% Ausbeute.



Schema 1: Schema der Totalsynthese von Aurantiosid G (**73**).

Schema 1 zeigt noch einmal die erste Synthese von Aurantiosid G (**73**) als natürlicher *N*-glykosylierter 3-Acyltetramsäure über insgesamt 22 Stufen.

Darauf aufbauend wurde im zweiten Abschnitt dieser Arbeit eine Reaktionssequenz zur Darstellung von Ancorinosid A (**75**) erarbeitet. Im Gegensatz zu der bereits bekannten Totalsynthese von Virgineon (**18**), einer natürlichen O-monoglykosidischen 3-Acyltetramsäure, sind Ancorinoside aus einem O-diglykosidischen Baustein aufgebaut. Sie stellen die ersten bekannten Tetramsäuren dieser Art dar, welche zusätzlich auf D-Asparaginsäure, einer nicht proteinogenen Aminosäure, basieren.

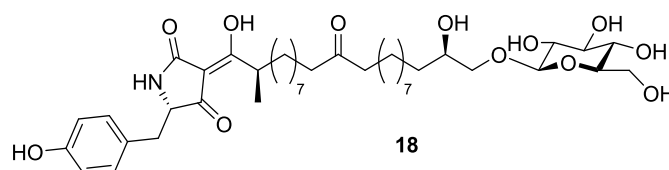
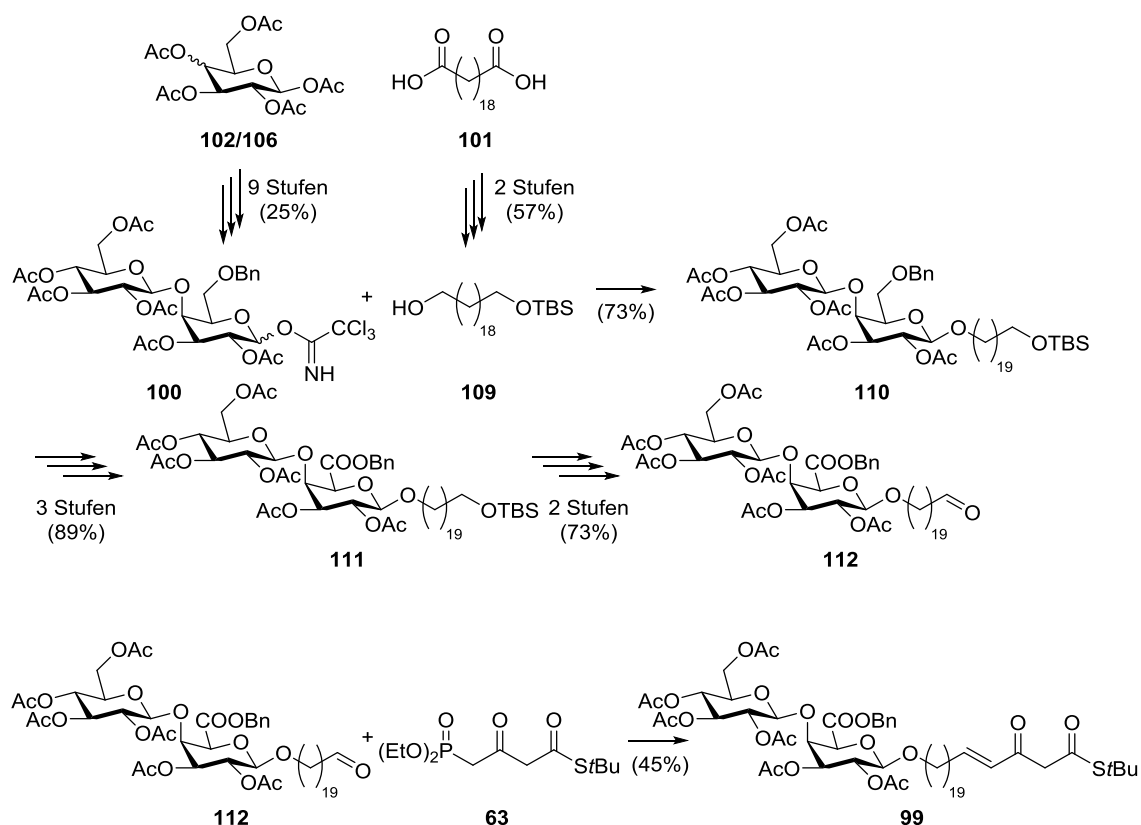


Abbildung 2: Struktur des monoglykosidischen Virgineon (**18**).

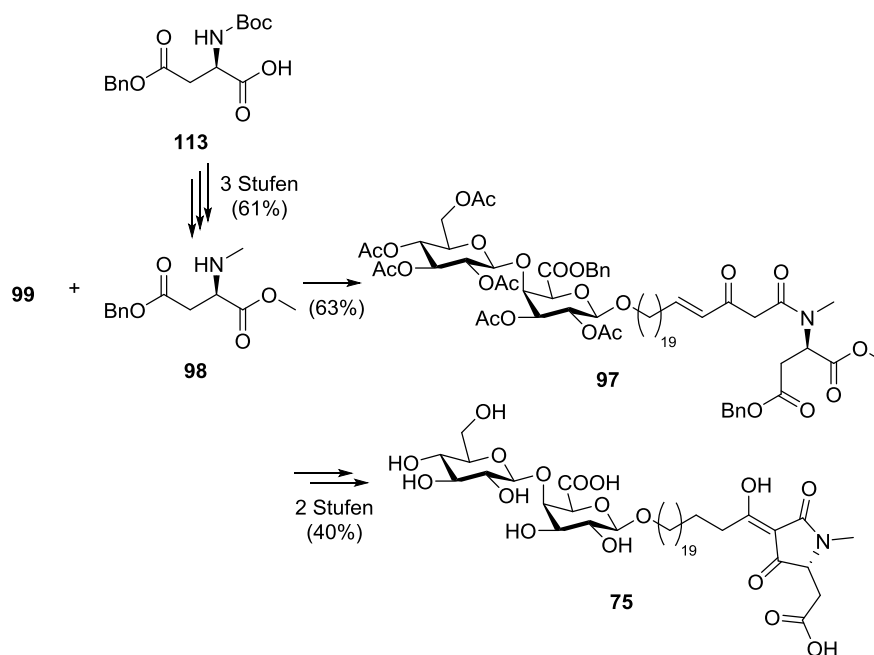
Anders als in der Synthese von Aurantiosid G wurde **75** sukzessive, startend mit dem Disaccharidfragment, aufgebaut. Für die Darstellung von Ancorinosid A wurde hierzu der diglykosidische Donor **100** über neun Stufen mit einer Ausbeute von 25%, aus den beiden kommerziellen, peracetylierten Monosacchariden D-Galactose (**102**) und D-Glucose (**106**) als erster Teilbaustein, erhalten. Dieser Imidat-Donor **100** wurde anschließend, unter Schmidt Bedingungen, an den Spacer **109** glykosyliert. Dieser Akzeptor **109** konnte in nur zwei Stufen aus Eicosandisäure (**101**) erhalten werden. Das glykosylierte Produkt **110** wurde nach Debenzylierung, Oxidation zur entsprechenden Galacturonsäure und Veresterung in das Uronat **111** überführt. Abspaltung der Silylschutzgruppe mit darauffolgender Oxidation ergab den Aldehyd **112**, welcher erfolgreich per HWE Olefinierung mit dem Thioesterphosphonat **63** zum Schlüsselfragment **99** umgesetzt werden konnte.



Schema 2: Darstellung des β -Ketothioesters **99**.

Zur Darstellung des Precursors **97** wurde der β -Ketothioester **99** mit dem *N*-Methylaminosäureester **98** kondensiert. Dieser Aminosäureester konnte in drei Stufen über 61% aus dem kommerziellen Aminosäurederivat Boc-D-Asp(OBn)-OH

(**113**) durch Schutzgruppenmodifikation und *N*-Methylierung erhalten werden. Eine hydrogenolytische Reduktion der Doppelbindung mit simultaner Benzylesterspaltung lieferte die direkte Vorstufe zu **75**, welche schließlich in einer simultan ablaufenden Lacey-Dieckmann-Zyklisierung und globalen Acetylsplaltung in das Ancorinosid A (**75**) überführt wurde.

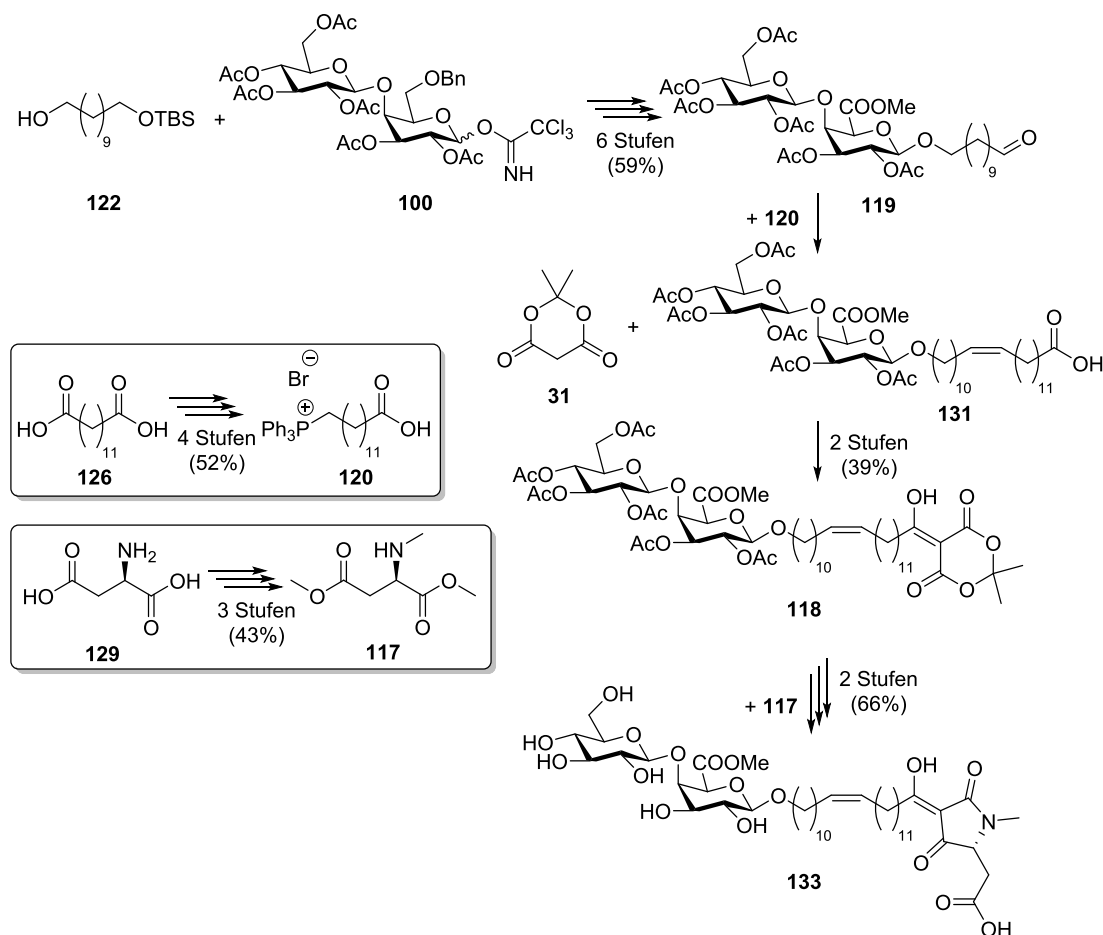


Schema 3: Totalsynthese von Ancorinosid A (**75**) via Aminolyse und Lacey-Dieckmann-Zyklisierung.

Über die in Schema 2 und 3 gezeigten Synthesesequenzen war es möglich Ancorinosid A (**75**) über 25 Stufen als erste *O*-diglykosidische 3-Acyltetramsäure darzustellen.

Im dritten Teil der Arbeit wurde, mit der Darstellung des Derivats Ancorinosid D (**76**), die Flexibilität der zuvor verwendeten Methode gezeigt. Hierbei wurde die Syntheseroute von **75** durch Modifikationen soweit wie nötig abgeändert. Ausgehend von dem bereits verwendeten Donor **100** wurde analog zur Synthese von **75** der „verkürzte“ Aldehyd **119** über insgesamt sieben Stufen dargestellt. Dieser konnte mit der zweiten Hälfte der Seitenkette unter Einbau der *Z*-Doppelbindung via Wittig Olefinierung mit dem Phosphoniumsalz **120** verknüpft werden. Eine Einführung des β -Ketoamid-Motivs erfolgte durch Acylierung der erhaltenen Carbonsäure an Meldrumsäure (**31**), als C-2 Baustein. Die Aminolyse zwischen **117** und **118** verlief mit einer simultanen Lacey-Dieckmann-Zyklisierung. Somit war es möglich direkt die vollgeschützte Form des natürlichen Ancorinosid

D (**76**) als Vorstufe in nur einer Reaktion zu synthetisieren. In einer globalen Verseifung wurde, entgegen der Erwartungen, das Methyluronat **133** als Derivat des natürlichen Ancorinosid D (**76**) erhalten (siehe Schema 4).



Schema 4: Synthesesequenz des Methyluronats **133**.

Zusammenfassend war es möglich den ersten synthetischen Zugang zur Klasse der halogenierten *N*-glykosylierten 3-Polyenoyltetramsäuren zu schaffen. Hierdurch konnte die erste Totalsynthese des natürlichen Aurantosid G (**73**) realisiert werden. Ebenso konnte ein flexibler Zugang zur Klasse der *O*-diglykosidischen 3-Acyltetramsäuren geschaffen werden, wodurch die Erstsynthese von Ancorinosid A (**75**) und Methyl-Ancorinosid D (**133**) erreicht werden konnte.

Summary

Object of this thesis was the synthesis of natural 3-acyltetramic acids. Tetramic acids and their C-3 acylated derivatives are an in nature often occurring structural motif. Many natural products with this motif show interesting biological activities, like antibiotic, antiviral or cytotoxic effects. As a result, 3-acyltetramic acids have been frequently studied natural products of scientific research and have come to the fore of many scientists. Although many natural 3-acyltetramic acids have already been analysed and synthesised, no general synthesis is known. Particularly in the group of glycosylated tetramic acids only few derivatives have been studied synthetically and biologically.

With this starting point in mind, it was intended to synthesise novel *O*- and *N*-glycosylated 3-acyltetramic acids. An additional goal was to create a facile modular access to this group. By this an easy derivatisation of the molecules and the synthesis of not only single products would be possible.

First part was the synthesis of *N*-glycosylated 3-acyltetramic acids. Besides the in the literature described streptolydigin (**20**), only the group of rubrosides (**74**) and aurantosides are known as natural *N*-glycosylated 3-acyltetramic acids. In this thesis it was possible to synthesise aurantoside G (**73**) as the first member of *N*-glycosylated 3-oligoenoyltetramic acids.

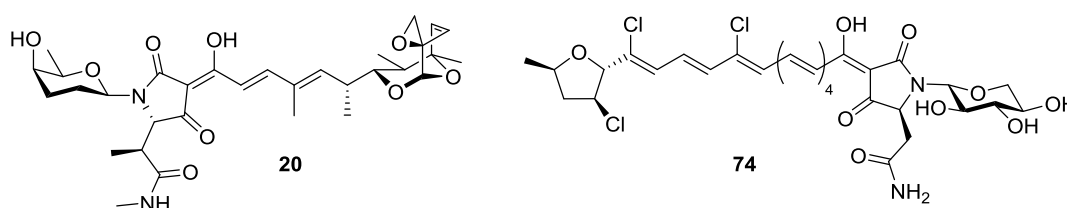
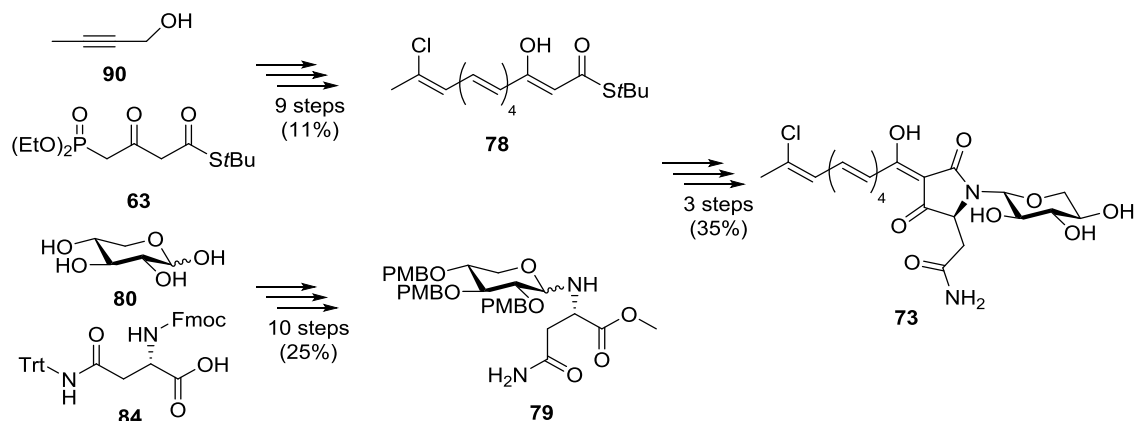


Figure 1: Structure of the *N*-glycosylated 3-acyltetramic acids streptolydigin (**20**) and rubroside H (**74**).

73 has been built out of two fragments, the halogenated and conjugated sidechain **78** and the *N*-glycosylated L-asparaginate **79**. **78** has been synthesised starting with the commercially available C-4 building block **90** by a gradually elongation using tandem-oxidation-Wittig reactions with an overall yield of 11% in nine steps. The *N*-glycoside **79** has been synthesised with an overall yield of 25% over ten steps starting from D-xylose (**80**) and the commercially available, L-asparagine derivative **84**. Condensation of **78** and **79** in a silver mediated aminolysis

fortunately only yielded the β -epimer. A concluding cleavage of the protection-groups followed by a Lacey-Dieckmann-cyclisation enabled the synthesis of aurantoside G (**73**) in three further steps in 35% yield.



Scheme 1: Scheme of the total synthesis of aurantoside G (**73**).

Scheme 1 shows the first total synthesis of **73** a natural *N*-glycosylated 3-acyltetramic acid over 22 steps.

Based on these results a synthetic route for the synthesis of ancorinoside A (**75**) was developed in the second part of this thesis. Unlike to the already synthesised natural monoglycosidic virgineone (**18**) ancorinosides embody an *O*-diglycoside and are the first known 3-acyltetramic acids containing this together with a non-proteinogenic D-amino acid.

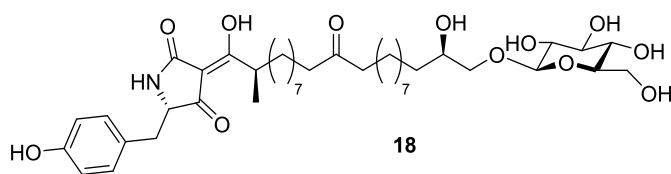
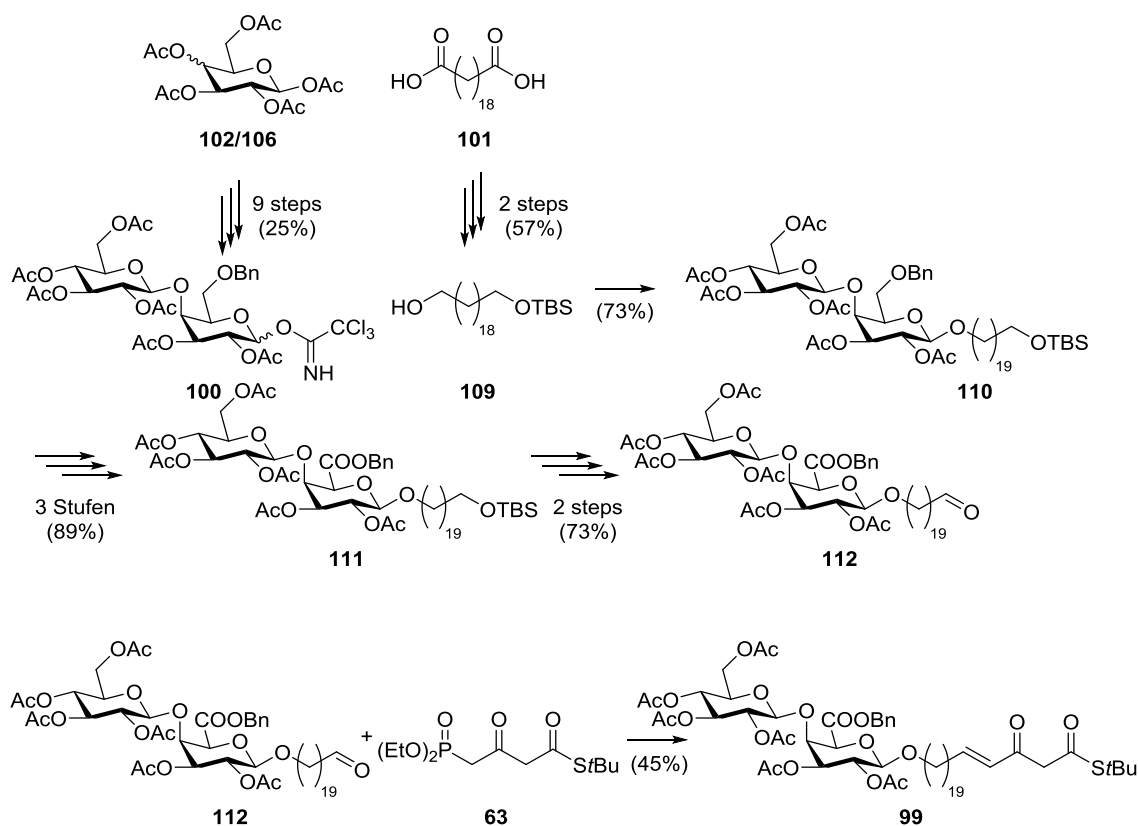


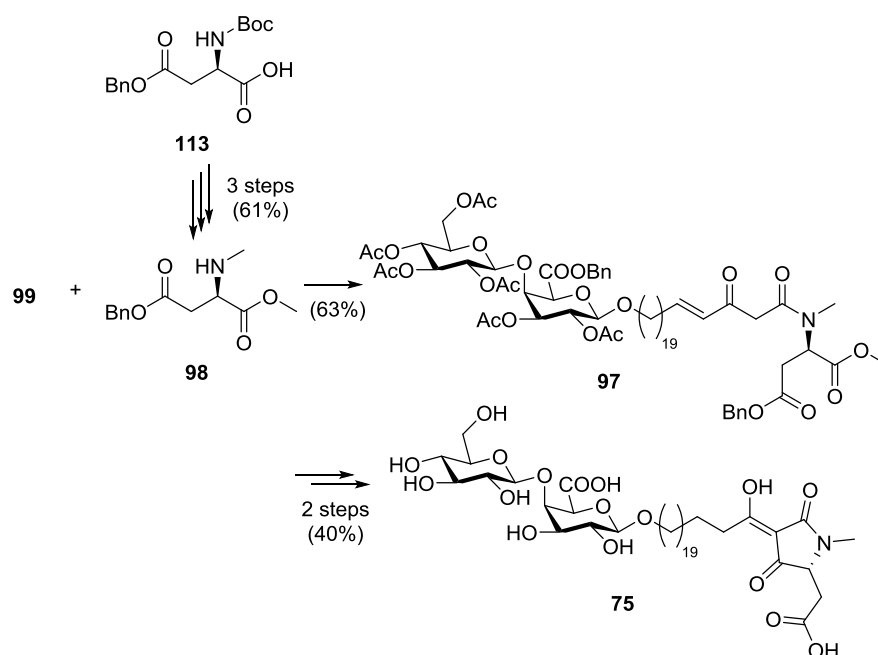
Figure 2: Structure of monoglycosidic virgineone (**18**).

Unlike to the synthesis of **73**, **75** has been built gradually starting from the diglycoside. For the synthesis of ancorinoside A the donor **100** has been prepared over nine steps with an overall yield of 25% starting from the commercially available peracetylated D-glucose (**106**) and D-galactose (**102**). This imidate **100** has been glycosylated to the spacer **109** using Schmidt-conditions. We synthesised acceptor **109** in two steps starting from eicosanedioic acid (**101**). Debenzylation of **110**, oxidation to the galacturonic acid and esterification resulted in the uronate **111**. Cleavage of the silyl group and subsequent oxidation led to

aldehyde **112** which was successfully olefinated with **63** resulting in the key fragment **99**.



For the synthesis of precursor **97** the β -ketothioester **99** was coupled with the *N*-methyl asparaginate **98**. This amine has been built in three steps in an overall yield of 61% from the commercial available amino acid derivative Boc-D-Asp(OBn)-OH (**113**). Hydrogenolytic reduction of the double bond in **97** with simultaneous debenzoylation afforded the direct pre-stage of **75** which was converted to ancorinoside A (**75**) in a Lacey-Dieckmann-cyclisation with simultaneous global acetyl cleavage.



Scheme 3: Totalsynthesis of ancorinoside A (**75**) via aminolysis and Lacey-Dieckmann reaction.

It was possible to synthesize ancorinoside A (**75**) using the route shown in Scheme 2 and Scheme 3 over 25 steps as the first *O*-diglycosidic 3-acyltetramic acid.

In the third part of this dissertation the flexibility of the above shown synthetic route was demonstrated using this sequence for the synthesis of the derivative ancorinoside D (**76**) with small changes. Based on the already used donor **100** the “shorter” aldehyde **119** was synthesized over seven steps analogously to **75**. This aldehyde was coupled with the second half of the sidechain with simultaneous installation of the double bond via a Wittig olefination by using phosphonium salt **120**. The introduction of the β -keto motif was realized by coupling of the obtained carboxylic acid with meldrum’s acid (**31**) as a C-2 building block. Aminolysis of **117** and **118** resulted in a concurrently running Lacey-Dieckmann-cyclisation. Thus, preparation of the fully protected natural ancorinoside D (**76**) was obtained in only one step. A global ester cleavage resulted contrary to expectation in the formation of methyl uronate **133** a derivative of natural ancorinoside D (**76**) (see Scheme 4).

Abkürzungsverzeichnis

| | |
|---------------------------|--|
| A | Adenylierungsdomäne |
| Ac | Acetyl- |
| AcMe | Aceton |
| ACP | Acyl carrier protein |
| All | Allyl- |
| AT | Acyltransferase |
| BAIB | Diacetoxiodbenzol |
| Bn | Benzyl- |
| Boc | <i>tert</i> -Butoxycarbonyl- |
| <i>n</i> BuOH | <i>n</i> -Butanol |
| C | Kondensationsdomäne |
| CAN | Cer(IV)ammoniumnitrat |
| CoA | Coenzym A |
| DCC | <i>N,N'</i> -Dicyclohexylcarbodiimid |
| DDQ | 2,3-Dichlor-5,6-dicyano-1,4-benzochinon |
| DH | Dehydratase |
| DIAD | <i>N,N'</i> -Azodicarbonsäurediisopropylester |
| DIBAL-H | Diisobutylaluminiumhydrid |
| DIPEA | Diisopropylethylamin |
| DMAP | 4-(Dimethylamino)pyridin |
| DMF | <i>N,N</i> -Dimethylformamid |
| DMP | Dess-Martin-Periodinan |
| EDCI | <i>N</i> -Ethyl- <i>N'</i> -(3-dimethylaminopropylcarbodiimid) hydrochlorid |
| ER | Enoylreduktase |
| Et | Ethyl- |
| EtOAc | Essigsäureethylester |
| Fmoc | 9-Fluorenylmethoxycarbonyl- |
| HWE | Horner-Wadsworth-Emmons |
| IPCF | Isobutylchloroformat |
| KO ^{<i>t</i>} Bu | Kalium- <i>tert</i> -butanolat |

| | |
|----------------|--|
| KR | Ketoreduktase |
| KS | Ketoacylsynthase |
| Me | Methyl- |
| MeCN | Acetonitril |
| Mp | <i>para</i> -Methoxyphenyl- |
| MS | Molekularsieb |
| MTT | 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromid |
| NaHMDS | Natrium bis(trimethylsilyl)amid |
| NCS | <i>N</i> -Chlorsuccinimid |
| NaOMe | Natriummethanolat |
| NDP | Nucleotid-diphosphat |
| NIS | <i>N</i> -Iodsuccinimid |
| NMR | Kernspinresonanz (<i>engl. nuclear magnetic resonance</i>) |
| NRPS | nicht ribosomale Peptidsynthetase |
| <i>o</i> Ns | <i>ortho</i> -Nosyl- |
| PCP | Peptidyl carrier protein |
| perOAcGlc | 2,3,4,6-Tetra- <i>O</i> -acetyl- β -D-glucopyranosyl- |
| Ph | Phenyl- |
| PKS | Polyketidsynthase |
| PMB | <i>para</i> -Methoxybenzyl- |
| PS | Polystyrol |
| <i>p</i> TosOH | <i>para</i> -Toluolsulfonsäure |
| quant | quantitativ |
| Red-Al | Natrium-bis(2-methoxyethoxy)aluminium dihydrid |
| rt | Raumtemperatur |
| TBAF | Tetrabutylammoniumfluorid |
| TBS | <i>tert</i> -Butyldimethylsilyl- |
| <i>t</i> Bu | <i>tert</i> -Butyl- |
| TEMPO | 2,2,6,6-Tetramethylpiperidin-1-oxyl |
| TFA | Trifluoressigsäure |

| | |
|--------|--------------------------------------|
| TfOH | Trifluormethansulfonsäure |
| THF | Tetrahydrofuran |
| TMSOTf | Trimethylsilyltrifluormethansulfonat |
| Trt | Trityl |

1 Einleitung

Sekundärmetabolite, auch allgemein als Naturstoffe bezeichnet, sind im Gegensatz zu den Primärmetaboliten wie Aminosäuren, Fettsäuren und Sacchariden, weder für die Entwicklung, den Aufbau noch die Lebenserhaltung eines Organismus verantwortlich. Stattdessen sind sie evolutionär aus der Anpassung des jeweiligen Lebewesens an die vorliegenden Umweltbedingungen, als Abwehrstoff gegen Fressfeinde oder auch im Falle von Tieren als Giftstoff zur Jagd entstanden.^[1-3]

Neben diesen Eigenschaften weisen viele Naturstoffe aber auch für den Menschen nützliche Wirkungen auf. Über die Jahrhunderte hinweg haben sich unzählige Verbindungen als Heilmittel erwiesen. In den Anfangszeiten wurden diese noch als direkte Rohextrakte, ohne genaue Kenntnisse über die eigentliche Zusammensetzung verwendet. So wurden beispielsweise bereits vor etwa 3000 Jahren Öle der Zypresse oder der Zeder von den Mesopotamiern gegen Husten und Entzündungen eingesetzt.^[4] Erst seit Beginn des 19. Jahrhunderts, mit dem Einzug verbesserter Analysemethoden, kam es zur Isolation und der genauen Bestimmung der eigentlich wirksamen Inhaltsstoffe. So wurde beispielsweise Morphin (**1**) (siehe Abbildung 3), vermutlich einer der ersten isolierten bioaktiven Naturstoffe, bereits 1805 von dem deutschen Apotheker Friedrich Sertürner aus der Milch des Schlafmohns *Papaver somniferum* isoliert.^[5,6] Dieses starke natürliche Analgetikum kam bereits damals ebenso wie auch heute noch immer in operativen Eingriffen sowie zur Schmerzbehandlung zum Einsatz.^[7] Ein ähnlich komplexes Beispiel für einen pharmakologisch verwendeten Naturstoff stellt das endozyklische Peroxid Artemisinin (**2**) dar. Dieses wird seit Ende der 80er Jahre zur Behandlung von Malaria eingesetzt. Die Pflanze *Artemisia annua* findet in der traditionellen chinesischen Medizin aber schon länger Anwendung in der Behandlung von Fieber und Erkältungen.^[1,4]

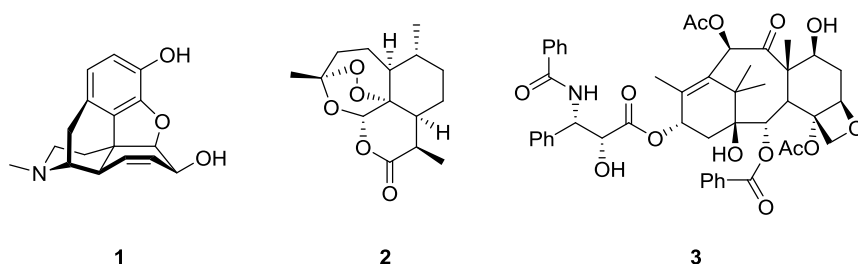


Abbildung 3: Strukturen von Morphin (1), Artemisinin (2) und Taxol (3) als Beispiele pharmazeutisch verwendeter Naturstoffe.

Anhand allein dieser ausgewählten Verbindungen ist zu sehen, wie häufig komplexe Strukturen, mit einer Vielzahl an Stereozentren, Ringsystemen und einer Fülle an Substituenten bzw. Heteroatomen in Naturstoffen aufzufinden sind. Diese diffizilen Strukturen sind oft charakteristisch für einen bestimmten Organismus.^[1,8,9] Mit dieser Fülle an Diversität stellen Naturstoffe einen idealen Ausgangspunkt für die Suche neuer Wirkstoffe und Pharmakophore dar. Dies zeigt sich am anschaulichsten an der Anzahl an zugelassenen Medikamenten. Bis Anfang der 90er Jahre beispielsweise waren etwa 80% aller auf dem Weltmarkt befindlichen Wirkstoffe direkte oder von Naturstoffen abgeleitete Verbindungen.^[4,10] Doch auch neuere Zahlen belegen diesen anhaltenden Trend. In den Jahren zwischen 2005 und 2007 kam es zur Zulassung 13 neuer Naturstoff-abgeleiteter Wirkstoffe.^[11] Zusätzlich sind über 100 Verbindungen mit dem Ursprung eines Naturstoffes in klinischen Studien der Phase 0 bis II als potentielle Medikamente.^[12]

Der Zugang zu Naturstoffen ist aber häufig durch ihre schlechte Verfügbarkeit oder die geringen Mengen an isolierbarem Material stark erschwert. So führte die Gewinnung des, aus der Rinde der pazifischen Eibe isolierten, Taxol (3) zu einem starken Rückgang der Pflanzenpopulation. Mit einer Ausbeute von etwa 0,01% läge der jährliche Bedarf bei tausenden von Bäumen, die nur langsam wachsenden Eibe, um die nötigen Mengen an 3 zu gewährleisten.^[13,14]

Um diese Problematik teilweise zu umgehen und eine größere Menge an Substanz zugänglich zu machen, hat sich das Gebiet der Naturstoffsynthese als eine der möglichen Alternativen angeboten. Diese hat sich mitunter von der reinen Bestätigung der Struktur der isolierten Metabolite hin zur Darstellung einer verwertbaren Menge entwickelt.^[15] Mit der Anwendung genau zugeschnittener Retrosynthesen war es möglich, größere Mengen an Naturstoffen mit einer möglichst eleganten und kurzen Reaktionssequenz zu erhalten.^[16] Zusätzlich hat sich die Naturstoffsynthese aber auch als nützliche Hilfe zur Untersuchung sowohl

des Naturstoffes sowie Derivaten davon ergeben. Mit Hilfe der Synthese können durch Vorstufen und Derivatisierungen Struktur-Leitmotive und Pharmakophore identifiziert werden um somit auch Struktur-Wirkungs-Beziehungen zu verstehen. So kann es sein, dass die Wirkungen durch Abänderung oder Vereinfachung der Struktur gesteigert und Nebenwirkungen vermindert werden können. Eine solche Derivatisierung der Strukturen ist im natürlichen Organismus, durch die Notwendigkeit der Abänderung der Proteinstruktur, nur schwer realisierbar. Durch die jahrelange Forschung an Strukturen von Naturstoffen konnten auf diesem Weg unzählige Leitmotive von biologisch interessanten Verbindungen herausgearbeitet werden. Ein Beispiel dafür ist das heterozyklische Pyrrolidin-2,4-dion System der Tetramsäuren. Das breite Spektrum an Bioaktivitäten und die Vielzahl verschiedener Naturstoffe in terrestrischen und marinen Lebensformen mit diesem Motiv macht Tetramsäuren seit Jahrzehnten ein von vielen Gruppen untersuchtes Ziel der akademischen Forschung.^[17–19]

1.1 Eigenschaften von Tetramsäuren

Der Heterozyklus der Tetramsäuren, welcher von einer Vielzahl von Bakterien, Pilzen und Schwämmen produziert wird, ist seit Anfang des 20. Jahrhunderts bekannt. Jedoch waren die biologischen Wirkungen dieser Struktur dabei noch völlig unerforscht. Erst nach Isolation und Charakterisierung einer Vielzahl von Naturstoffen wurde die Bedeutung der Tetramsäuren ab etwa 1960 richtig erkannt. Viele Strukturen besaßen eine antibiotische, antiviral, antimykotische oder zytotoxische Wirkung. Tetramsäuren können in zwei tautomeren Formen, der Diketo- **4a** oder der Enolform **4b** vorliegen (siehe Abbildung 4). Die Pyrrolidin-2,4-dion (**4a**) Form stellt jedoch die im Gleichgewicht meist überwiegende Tautomerenform dar.

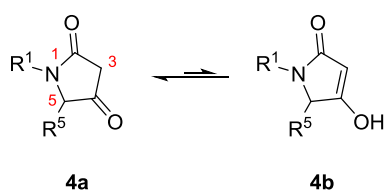


Abbildung 4: Allgemeine tautomere Strukturen von 3H-Tetramsäuren **4a/4b** mit deren Nummerierung (rot).

In der Natur kommen Tetramsäuren jedoch am häufigsten in Form ihrer an Position C-3 acylierten Derivate vor. Diese 3-Acyltetramsäuren bilden die Hauptvertreter der bioaktiven Tetramsäure-Derivate. 3-Acyltetramsäuren liegen in einem komplexen tautomeren Gleichgewicht von bis zu neun Formen vor. In vielen Fällen können jedoch nur vier der Tautomere beobachtet werden. Diese lassen sich in je zwei Paare aus inneren tautomeren Formen **5a/b** und **5c/d** und zwei Paaren aus äußeren **5a/c** und **5b/d** unterteilen (siehe Abbildung 5). Aufgrund der schnellen Übertragung der Enol-Protonen ist es nicht möglich, die inneren Paare **5a/b** bzw. **5c/d** per NMR zu unterscheiden. Die äußeren Paare **5a/c** bzw. **5b/d** können jedoch, in Folge der langsamen Rotation um eine C-C Bindung, voneinander unterschieden werden. Das Verhältnis der auftretenden Tautomere ist dabei sowohl von den Substituenten am Stickstoff und Position C-5 sowie der verwendeten Isolationsmethode abhängig und kann deshalb nicht genau vorhergesagt werden. Jedoch wird im Allgemeinen die Struktur **5a** als häufigste Form beobachtet.^[20–22]

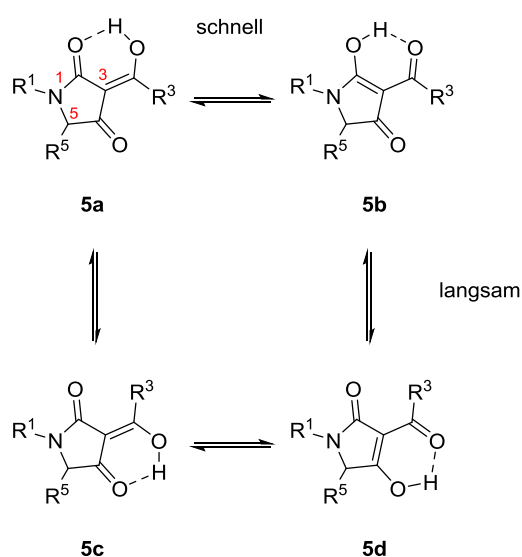


Abbildung 5: Allgemeine Strukturen der vier Hauptformen von 3-Acyltetramsäuren **5a-d** mit enthaltener Nummerierung (rot).

In Folge dieser tautomeren Strukturen, ist es 3-Acyltetramsäuren möglich, als Chelatoren für diverse zwei- und dreiwertige Metallkationen zu dienen und zum Teil sehr stabile Metallkomplexe auszubilden.^[23,24] Beispielsweise wurden Magnesidin A (**6**) oder auch Tenuazonsäure (**7**) nur als entsprechende Ca^{2+} bzw. Mg^{2+} Komplexe isoliert.^[25,26] In Analogie zur scheinbaren Stabilität der Enolstruktur **5a**, werden Metallionen hauptsächlich mittels 3-Acylsauerstoff und dem C-2

Carbonyl-Sauerstoff-Atom komplexiert. Es wird vermutet, dass eine solche Komplexierung zur Steigerung der Stabilität des eigentlichen Naturstoffs beiträgt. So war es beispielsweise nicht möglich Geodin A (**8**) aus seinem Magnesiumsalz freizusetzen und dieses stabil als freie Säure zu isolieren.^[27] Es wird außerdem diskutiert, dass erst die Metallkomplexe für bestimmte pharmakologische Wirkungen verantwortlich sind. So geht die inhibitorische Wirkung von Harziansäure (**9**) gegenüber der Serin/Threonin Phosphatase Type 2A in Abwesenheit von Zn^{2+} Ionen vollständig verloren.^[28] Zusätzlich Weise steigern diese Metallkomplexe die Lipophilie der polaren Naturstoffe und ermöglichen so einen besseren Transport durch die Zellmembran, was ebenfalls ihrer biologischen Wirkung zugutekommt.^[17,27,29]

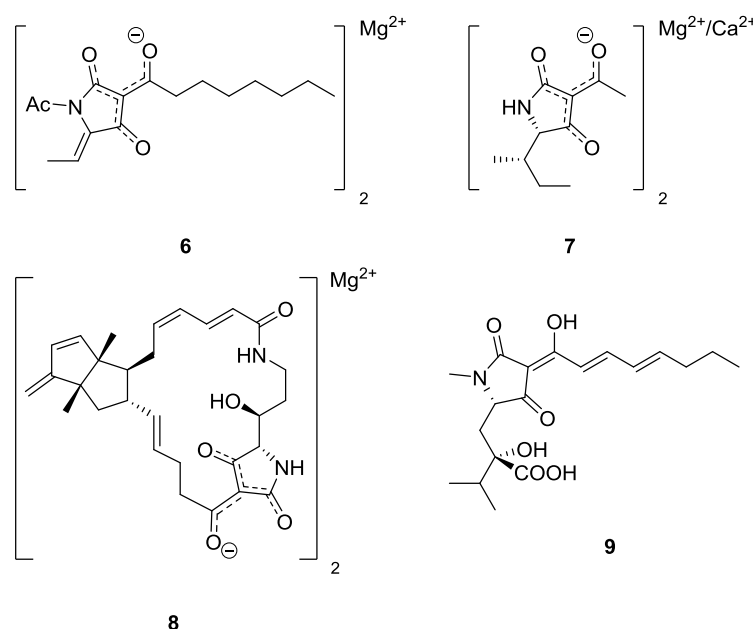
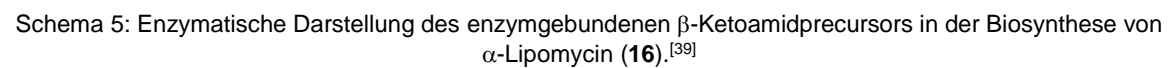


Abbildung 6: Strukturen der isolierten natürlichen 3-Acyltetramsäuren Magnesidin A (**6**), Tenuazonsäure (**7**), Geodin A (**8**) und Harziansäure (**9**).

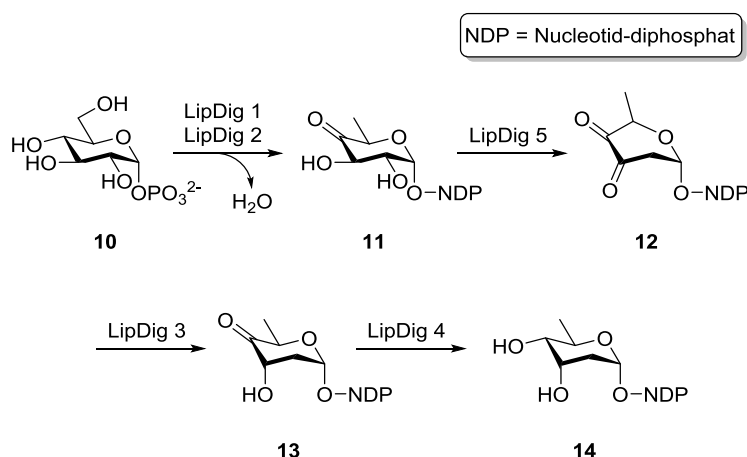
1.2 Biosynthese von 3-Acyltetramsäuren

Trotz der großen strukturellen Diversität von natürlichen 3-Acyltetramsäuren **5** kann deren biologischer Ursprung auf eine größtenteils gemeinsame Biosynthese zurückgeführt werden. Dabei sind **5** typische hybride Sekundärmetabolite und stammen aus Polyketiden und hauptsächlich aus natürlichen α -Aminosäuren.^[17] Diese werden über ein Typ I Polyketidsynthase (PKS) nicht ribosomale

Peptidsynthetase (NRPS) Hybrid aufgebaut und verknüpft. In diesem Prozess kommt es, analog zum bekannten Fettsäurebiosyntheseweg, in der PKS zum sequenziellen Aufbau der 3-Acylseitenkette. Ein Acyl-CoA-Fragment wird mittels Acyltransferase (AT) auf das Acyl carrier protein (ACP) übertragen und mittels β -Ketoacylsynthase (KS) durch Malonyl CoA oder Methylmalonyl CoA unter CO_2 Abspaltung verlängert. Nach jeder Verlängerung wird die erhaltene β -Ketothioestereinheit je nach Metabolit per Ketoreduktase (KR), Dehydratase (DH) und Enoylreduktase (ER) in ein gesättigtes Acylthioester Fragment überführt.^[30,31] Die vollständige Seitenkette wird abschließend von der PKS auf die NRPS übertragen. Dabei wird eine, als Thioester am Peptidyl carrier protein (PCP) gebundene, Aminosäure mittels Adenylierung in der A-Domain der NRPS aktiviert und schließlich in der C-Domain unter Kondensation und Ausbildung einer Amidbindung an das Polyketid übertragen.^[32–34] Das erhaltene 3- β -Ketoamidthioester-Kondensat wird abschließend unter Bildung der Tetransäure vom Enzymcluster abgespalten. Bis dato sind mehrere Möglichkeiten der Bildung des Heterozyklus bekannt und diskutiert.^[35–37] Beispielsweise kommt es, wie für Equisetin (**24**) gezeigt wurde, durch die reduktive Domäne (R-Domain) im terminalen Modul des PKS-NRPS Hybrids zur Katalyse einer Dieckmann-Zyklisierung.^[38] Zeitgleich wurde von der Gruppe um Gui *et al.* die erste Dieckmann-Cyclase identifiziert. In diesem alternativen Weg kommt es, wie am Beispiel von Tirandamycin B gezeigt, im Cytoplasma direkt in einer enzymatisch vermittelten Dieckmann Kondensation zur Ausbildung des Pyrrolidin-2,4-dion-Rings.^[37] Obwohl bereits mehr als 150 verschiedene natürliche Tetransäurederivate identifiziert wurden, konnte erst von etwa 20 Vertretern der genaue Biosyntheseweg aufgedeckt werden.^[17,37] Eines der ersten Beispiele ist die aufgeklärte Biosynthese des konjugierten und glykosylierten α -Lipomycins (**16**). Dieses zeigt im Einzelnen die beteiligten Enzyme des Typ I PKS-NRPS Hybrids. Im ersten Schritt kommt es im Beladungsmodul L zum Acyltransfer eines Isobutyl-CoA mit anschließender zweifacher Kettenverlängerung durch Methylmalonyl-CoA in den Modulen I und II, jedoch ohne die Beteiligung einer Dehydratase (DH) in Modul I. Eine viermalige Kettenverlängerung mittels Malonyl-CoA in den Modulen III bis VI liefert schließlich die Pentaenoyl-Acylkette. Das letzte Modul erzeugt auf Grund des Fehlens einer Ketoreduktase die abschließende

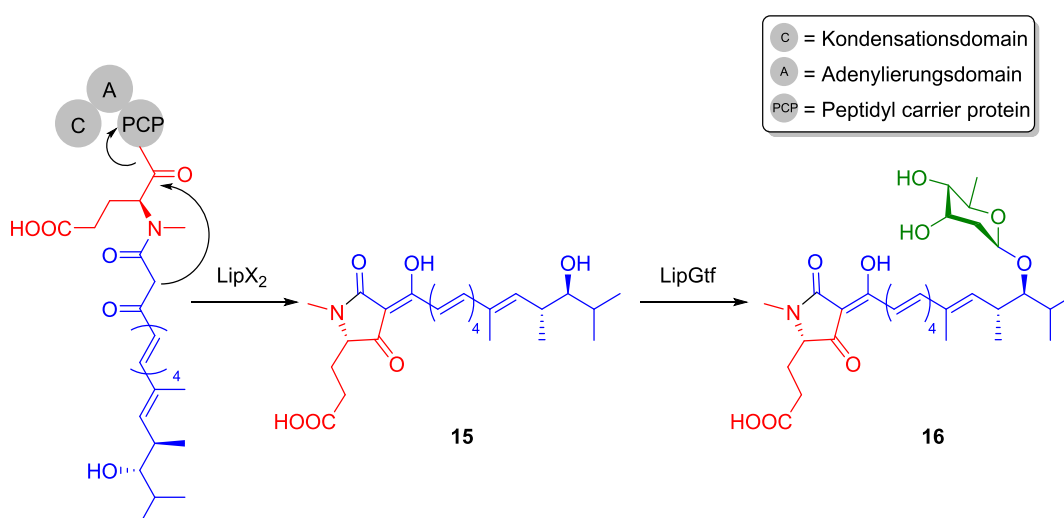


Desoxysaccharide zentrale Intermediat 1-NDP-4-Keto-6-deoxy- α -D-Glucose (**11**) überführt. Eine anschließende Oxidation an Position C-3 mit darauffolgender stereoselektiver Reduktion der beiden resultierenden Ketofunktionalitäten an Position C-3 und C-5 liefert das NDP α -gebundene D-Digitoxose Derivat **14** (siehe Schema 6).^[40]



Schema 6: Biosynthese von NDP gebundener D-Digitoxose (**14**).

In Schema 7 ist die, bereits durch biostatistische Analyse und totalsynthetische Darstellung bestimmte, absolute Konfiguration aller Stereozentren mit aufgenommen. In der Biosynthese wird nach Ringschluss via Dieckmann Cyclase zuerst β -Lipomycin (**15**) und nach Transglykosylierung, mit der NDP gebundenen D-Digitoxose an den sekundären Alkohol, das α -Lipomycin (**16**) gebildet.^[41–44]



Schema 7: Biosynthetischer Ringschluss von β -Lipomycin (**15**) und Glykosylierung zu α -Lipomycin (**16**).^[39]

Die gezeigte Biosynthese von α -Lipomycin (**16**) dient als anschauliches Beispiel eines typischen PKS-NRPS-Hybridweges via Dieckmann-Cyclase und verläuft analog zu denjenigen anderer aufgedeckter Strukturen wie dem Streptolydigin (**20**).

1.3 Natürliche 3-Acyltetramsäuren

Wie bereits in Kapitel 1.1 erwähnt wurde, ist eine große strukturelle Diversität in natürlichen 3-Acyltetramsäuren **5** möglich. Generell unterscheiden sich Tetramsäuren in ihrem von α -Aminosäuren abgeleiteten Rest durch Substituenten an Position C-5, Substituenten am Amidstickstoff und hauptsächlich in ihrer 3-Acylseitenkette. Die Vielfalt an Strukturen reicht dabei von einfachen gesättigten Seitenketten, oligoenoylen und polyenolyen Systemen, Decanoylresten, bis hin zu makrozyklischen Derivaten. Je nach Definition werden natürliche 3-Acyltetramsäuren in eine dieser Gruppen eingeordnet. Ein typischer Vertreter der Klasse der einfachen Tetramsäuren, ist neben der bereits gezeigten Tenuazonsäure (**7**), als einfachste bekannte natürliche 3-Acyltetramsäure mit nur einer Methylseitenkette, das Penicillenol B₁ (**17**). Doch auch substituierte Vertreter wie das Virgineon (**18**) werden ebenso zu dieser Gruppe gezählt. Dessen gesättigte Seitenkette ist endständig mit D-Mannose glykosyliert und kann zusammen mit dem bereits erwähnten α -Lipomycin (**16**) auch zusätzlich zur Untergruppe der natürlichen glykosylierten 3-Acyltetramsäuren gezählt werden. Doch auch hoch komplexe substituierte Derivate, wie das bereits 1955 isolierte Blasticidin A (**19**) zählen zu der Gruppe der einfachen Tetramsäuren.^[45] Die Strukturen der genannten einfachen 3-Acyltetramsäuren sind in Abbildung 7 noch einmal zusammen dargestellt.

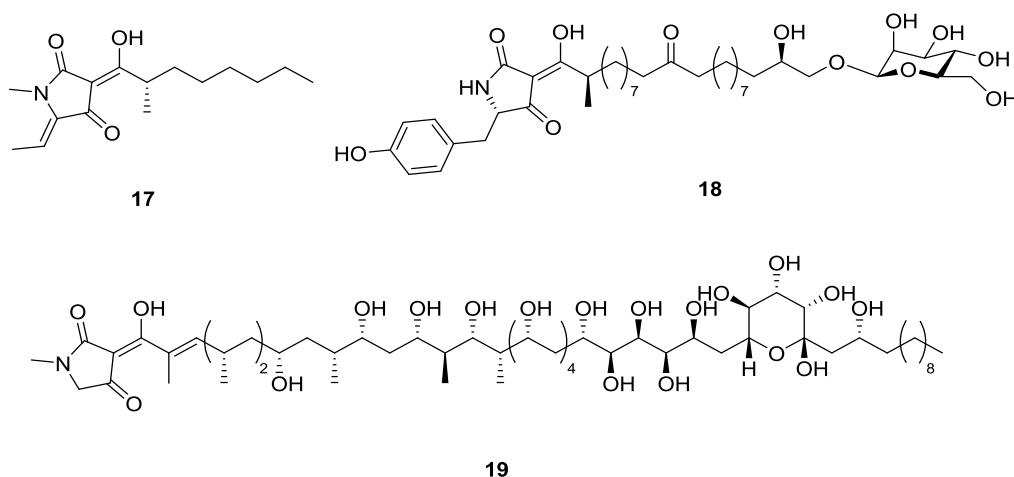


Abbildung 7: Strukturen von gesättigten 3-Acyltetramsäuren. Penicillenol B1 (**17**), Virgineon (**18**) und Blastidicin (**19**).

Ähnlich hierzu zeichnet sich die Gruppe der 3-Oligoenoyltetramsäuren durch eine lineare, aber konjugiert ungesättigte Seitenkette aus. Diese zeigen mit ihren zwei oder mehr Doppelbindungen häufig eine intensive Pigmentfarbe im entsprechenden Organismus und besitzen häufig eine außergewöhnliche bioaktive Wirkung. So wirkt das Streptolydigin (**20**) und ebenso dessen synthetische Derivate stark antibiotisch gegenüber Gram-positiven Bakterien mittels Inhibierung der bakteriellen RNS Polymerase.^[46–49] Ebenso zeigt Torrubellon D (**23**), ein dreifach ungesättigtes System, in Biotests eine antimalaria-aktive Wirkung und ebenfalls ein schwach antibakterielles Verhalten gegenüber *E. Coli* Δ TolC. Die absolute Konfiguration von natürlichem **23** konnte aber trotz Totalsynthese aller theoretisch möglichen Diastereomeren bis dato nicht bestimmt werden.^[50,51] Die stärkste bioaktive Wirkung zeigen die 3-Polyenoyltetramsäuren mit mehr als drei konjugierten Doppelbindungen. Bereits 1949 konnte Erythroskyrin (**22**) als eines der Pigmente des Pilzes *Penicillium islandicum* Sopp mit einer antibakteriellen Aktivität gegen diverse *Staphylococcus* Stämme isoliert werden. Somit stellt **22** die erste bekannte konjugiert ungesättigte 3-Acyltetramsäure dar.^[52,53] Die absolute Stereokonfiguration des komplexen Bizyklus konnte jedoch erst mehr als 30 Jahre nach Erstisolierung vollständig aufgeklärt werden.^[54,55] Ebenso stellt das vermeidlich einfachere Fuligorubin A (**21**), welches für die typische gelbe Farbe von Schleimpilzen (*fuligo septica*) verantwortlich ist, eine typische 3-Polyenoyltetramsäure dar.^[56,57] Während der Isolation dessen konnten die Autoren zusätzlich die Instabilität solcher konjugiert ungesättigter 3-Acyltetramsäuren zeigen. Während der Isolation von **21** war es

nötig auf Lichtausschluss ebenso wie auf eine ausreichende Kühlung der Rohextrakte während der Aufreinigung zu achten, um eine Zersetzung des Naturstoffs zu verhindern.

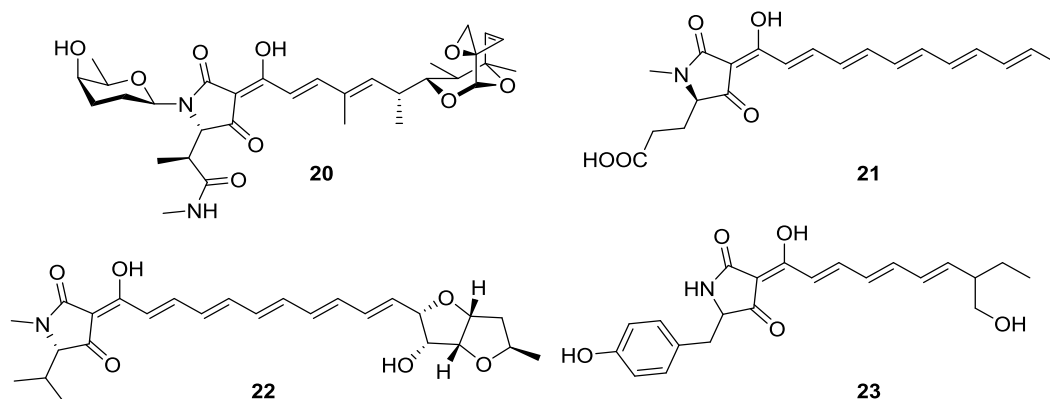


Abbildung 8: Strukturen ausgewählter 3-Oligo- und Polyenoyltetramsäuren. Streptolydigin (**20**), Fuligorubin A (**21**), Erythroskyrin (**22**) und Torribiellon D (**23**).

Der Vollständigkeit halber sollen auch die beiden weiteren großen Gruppen an natürlichen 3-Acyltetramsäuren kurz erwähnt werden. Hierunter fallen, die bereits erwähnten Decanoyltetramsäuren, mit mehr als 20 Vertretern, und die makrozyklischen Derivate, mit etwas mehr als 40 Vertretern. Das antibiotische und HIV inhibierende Equisetin (**24**), welches bereits seit 1979 bekannt ist, stellt mit seinem C-15 sesquiterpenoiden Ringsystem den ersten und wahrscheinlich am intensivsten untersuchten Vertreter dieser Gruppe dar.^[58,59] Biosynthetisch leiten sich 3-Decanoyltetramsäuren von den 3-Oligoenoyltetramsäuren ab und werden ausgehend von einer dreifach ungesättigt konjugierten Vorstufe mittels enzymatisch katalysierter Diels-Alder Reaktion biosynthetisch dargestellt.^[38,60] Die Tetramsäuren der Gruppe der makrozyklischen Derivate dagegen leiten sich allesamt von der Aminosäure Ornithin ab und bilden mit einem zusätzlichen Bi- oder Trizyklus in der Kette ein 16 oder 20 gliedriges Makrolactam (siehe **8** Abbildung 6 und **25** Abbildung 9).^[27,61–63] Zusätzlich sind drei nur wenig beschriebene Ausnahmen in der Literatur bekannt, welche sich in ihrem Ringsystem und oder der Kettenlänge des Makrolactams unterscheiden.^[64–66] Zu den Makrolactamen gehören auch die phytotoxischen Derivate Macrocidin A (**26a**) und B (**26b**), welche einen von Tyrosin abgeleiteten makrozyklischen Ether aufweisen.^[67]

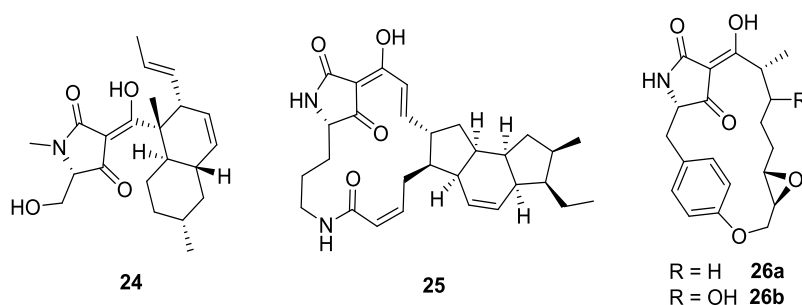


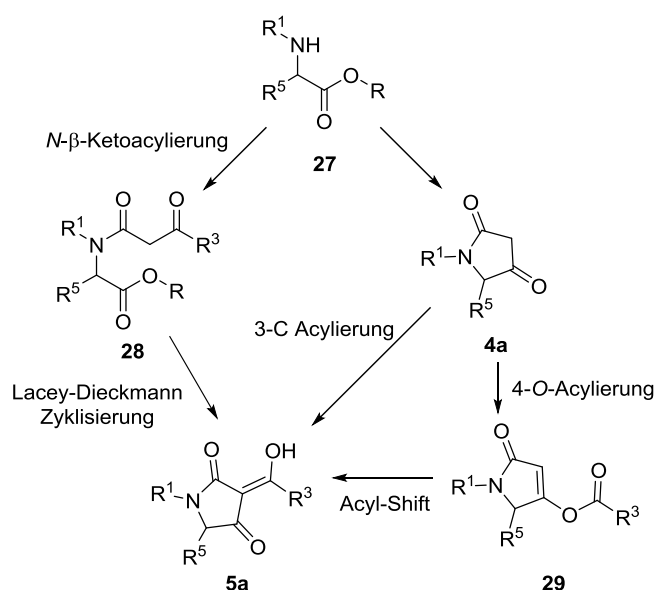
Abbildung 9: Beispielhafte Strukturen von Decanoyltetramsäuren und makrozyklischen Tetramsäuren. Equisetin (**24**), Ikarugamycin (**25**) und Macrocin A und B (**26a** und **26b**).

Neben diesen vier Gruppen von 3-Acyltetramsäuren sind noch *N*-acylierte Tetramate, Spirotetramsäuren oder an Position C-3 doppelt substituierte 3H-Tetramsäuren in der Natur aufzufinden, auf welche im Rahmen dieser Arbeit aber nicht näher eingegangen wird.

1.4 Chemische Darstellung von Tetramsäuren

Seit Isolation der ersten Vertreter natürlicher 3-Acyltetramsäuren wurden verschiedene Synthesewege für diese komplexen Strukturen entwickelt. Im Wesentlichen können zwei Optionen der Synthese von 3-Acyltetramsäuren unterschieden werden.

Auf der einen Seite steht die Synthese des Pyrrolidin-2,4-dions **4** an primärer Stelle. Dieser folgt die Einführung der 3-Acylseitenkette. Im zweiten Ansatz wird ein Vorläufer mit bereits enthaltener Seitenkette zum Heterozyklus, über eine sogenannte Lacey-Dieckmann-Zyklisierung, geschlossen. Beide Synthesewege sind in Schema 8 vereinfacht dargestellt.

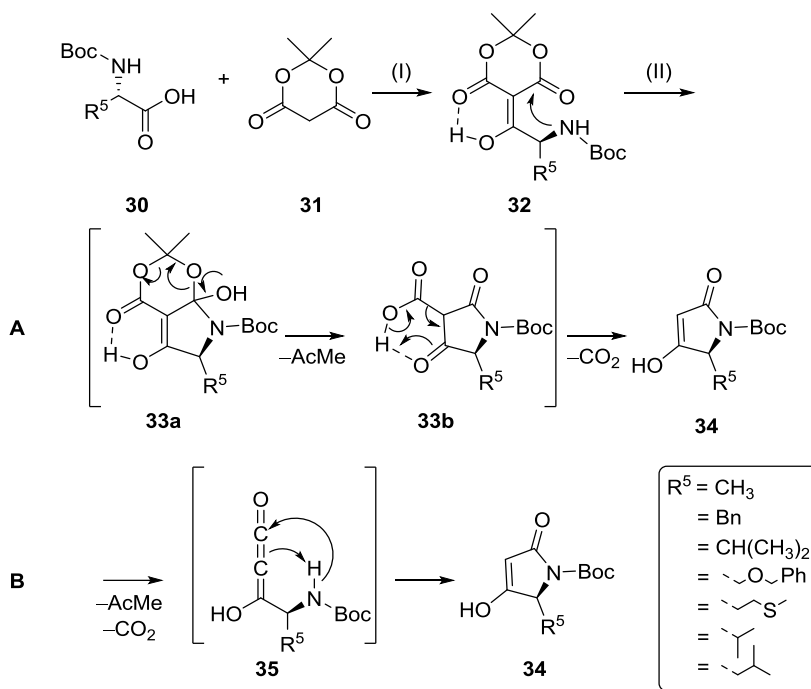


Schema 8: Mögliche Synthesewege zum Aufbau von 3-Acyltetramsäuren **5a**.

Alle Synthesestrategien greifen dabei auf α -Aminosäuren aus dem Chiral Pool als Ausgangsstoffe zurück. Je nach Reaktionsweg wird von der jeweils am Stickstoff geschützten Aminosäure ($\text{R}^1 \neq \text{H}$, $\text{R} = \text{H}$) oder einem Aminosäureester ($\text{R} \neq \text{H}$) ausgegangen. Nachfolgend werden kurz die jeweiligen Reaktionswege erläutert und deren Vor- und etwaige Nachteile erörtert.

1.4.1 Darstellung von Tetramsäuren über Meldrumsäureaddukte

Die erste allgemeine Methode zur Darstellung von **4a/4b** entwickelte die Gruppe um Jouin *et al.*^[68] Diese nutzte die bereits für die Darstellung von β -Ketoestern verwendete Meldrumsäure (**31**) zur Acylierung von Boc-geschützten Aminosäuren **30** mittels IPCF- und DMAP-Aktivierung unter Stereoretention. Das stabile acylierte Produkt **32** kann anschließend in einem thermischen intramolekularen Ringschluss unter Aceton und CO_2 -Abspaltung in die 3H-Tetramsäure **34** überführt werden. Schema 9 zeigt den von Jouin angenommen Mechanismus zur Bildung von Boc-geschützten Tetramsäuren **34** (Route A). Neuere Studien gehen von einer primären CO_2 -und Aceton-Abspaltung unter Bildung einer Ketenspezies **35** aus. Diese bildet anschließend in einem intramolekularen nucleophilen Angriff durch den Amid-Stickstoff die 3H-Tetramsäure **34** (Route B).^[69,70]



Schema 9: Vermutete Mechanismen der Darstellung von Boc geschützten Tetramsäuren **34** via Meldrumsäure (**31**).

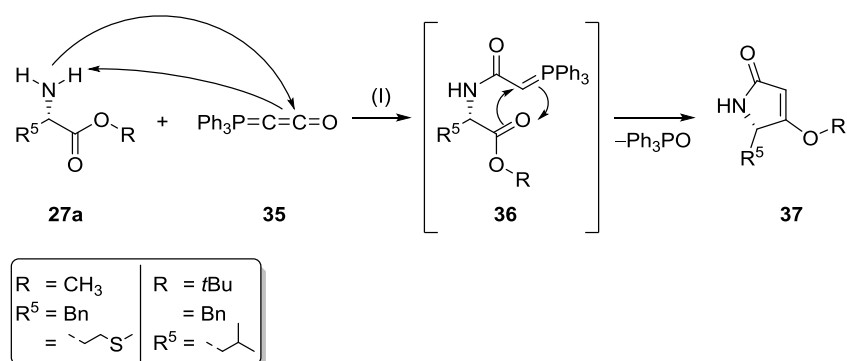
Reagenzien und Bedingungen: (I) DMAP, IPCF, CH_2Cl_2 , -5°C , 2 h; (II) EtOAc, reflux, 0,5 h.

Die Methode konnte durch Arbeiten der Gruppen um Joullie *et al.* und Ma *et al.* weiter verbessert werden.^[71,72] Diese ersetzten das sehr teure und giftige Aktivierungsreagenz IPCF durch DCC und änderten die Aufarbeitungsmethode ab um das stark aktivierte Acylmeldrumsäureprodukt **32** mit verbesserten Ausbeuten zu erhalten. Tønder *et al.* war es zusätzlich möglich die Abtrennung der als Nebenprodukt anfallenden Harnstoffderivate zu verbessern. Durch Verwendung von EDCI als Aktivator konnten die nun während der Reaktion gebildeten wasserlöslichen Harnstoffderivate durch einfache Extraktion entfernt werden.^[73] Mit der Verwendung dieser Methode war es nun möglich 3H-Tetramsäuren **4** unter Retention der Stereoinformation der ausgehenden α -Aminosäure darzustellen. Dieser Weg erlaubt aber leider nur die Synthese von *N*-geschützten 3H-Tetramsäuren, was eine weitere Modifizierung nötig macht um die gewollten 3-Acyltetramsäuren **5** darzustellen.

1.4.2 Darstellung via Domino-Addition-Intra-Wittig Reaktion

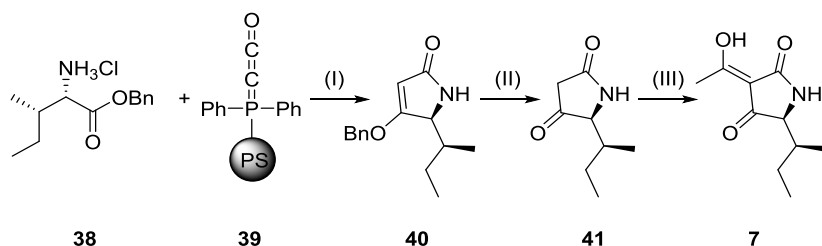
Die Gruppe um Schobert *et al.* entwickelte eine Synthesemethode zur Darstellung von Tetramaten **37** ausgehend von Aminosäureestern **27a** unter Verwendung des

stabilen Ketenylidentriphenylphosphorans (**35**) (siehe Schema 10).^[74–76] In der Reaktion kommt es nach Protonierung des Ylid C-Atoms zur Addition des freienamins an das Carbonyl C-Atom. Das aktivierte Amid-Ylid **36** reagiert anschließend unter Ringschluss in einer intramolekularen Wittig Olefinierung mit dem Estercarbonyl zum Tetramat **37**. Dieses kann, bei der Verwendung der entsprechenden Aminosäureester **27a**, leicht in die 3H-Tetramsäuren **4a/4b** überführt werden.^[77,78]



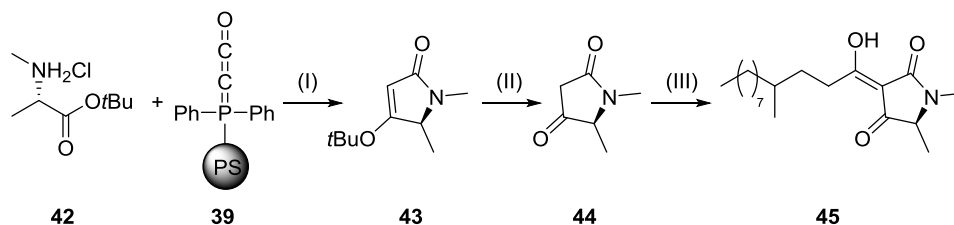
Schema 10: Allgemeiner Mechanismus zur Darstellung von Tetramaten **37** nach Schobert *et al.*
 Reagenzien und Bedingungen: (I) PhMe, reflux, 12 h.^[74,76,78]

Das Problem der Abtrennung des bei der Reaktion gebildeten Triphenylphosphinoxids konnte ebenfalls von der Gruppe um Schobert *et al.*, durch die Verwendung von Festphasen-gebundenem **35** bereinigt werden. Die einfache Darstellung von Tenuazonsäure (**7**) via **39** zeigte die Möglichkeiten welche diese Methode liefert (siehe Schema 11).^[77] In der Synthese wird aus dem Benzylester des L-Isoleucinbenzylesterhydrochlorids (**38**) mittels **39** das Benzyltetramat **40** erzeugt, welches durch hydrogenolytische Benzyl-Abspaltung die 3H-Tetramsäure **41** liefert. Dennoch ist, wie bei der Darstellung von **7** in Schema 11 zu sehen, bei der Verwendung des stabilen Ylids **35** ebenso wie bei der Verwendung von Meldrumsäure (**31**) eine nachträgliche Einbringung der 3-Acylseitenkette nötig.



Schema 11: Synthese von **7** via immobilisiertem Ylid **39**.
 Reagenzien und Bedingungen: (I) THF, 60 °C, 14 h; (II) H₂, Pd/C, MeOH, rt, 2 h; (III) BF₃ x Et₂O, AcCl, 80 °C, 8 h.^[77]

Auf diese Weise war es möglich auch *N*-methylierte Derivate wie die Melophrine A, B, C und G, über das Festphasen-gebundene Ylid **39** darzustellen. Schema 12 zeigt diese Synthese am Beispiel von Melophrin B (**45**).^[79]



Schema 12: Darstellung von Melophrin B (**45**) mit immobilisierten Ylid **39**.
Reagenzien und Bedingungen: (I) THF, 60 °C, 12 h; (II) TFA, rt, 3 h; (III) (a) $\text{BF}_3 \times \text{Et}_2\text{O}$, $\text{ClOC}(\text{CH}_2)_2\text{CH}(\text{CH}_3)\text{C}_8\text{H}_{17}$, 100 °C, 45 min; (b) MeOH, reflux, 2 h.^[79]

Neben diesen Methoden haben sich diverse weitere Möglichkeiten zum Aufbau von speziellen Tetramsäuresystemen oder des freien Tetramsäureheterozyklus entwickelt. Auf Grund der fehlenden Stereokontrolle oder der nur speziellen Anwendung werden diese in der vorliegenden Arbeit jedoch nicht beschrieben. Sie haben auch bis dato keine nennenswerten Anwendungen in Naturstoffsynthesen gefunden.^[80–84]

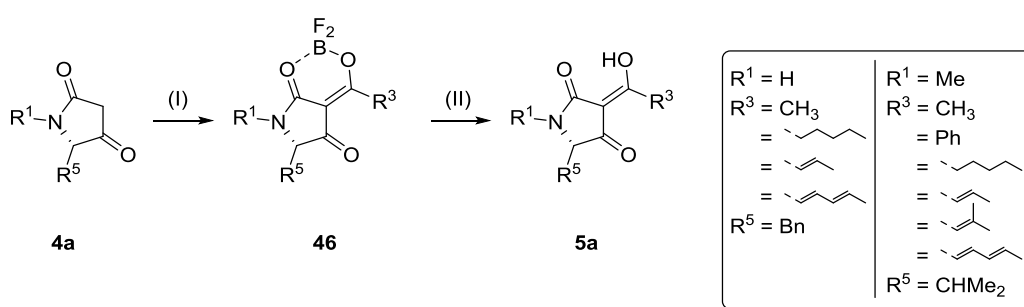
1.5 3-Acylierung von Tetramsäuren

Die in Kapitel 1.4.1 und 1.4.2 vorgestellten Methoden eignen sich zur generellen Darstellung von C-3 unsubstituierten Tetramsäuren **4a/4b** oder Tetramaten **37** (siehe Schema 10). Wie jedoch bereits gezeigt (siehe Kapitel 1.3) liegen natürliche Tetramsäuren hauptsächlich in einer an Position C-3 acylierten Form vor. Zusätzlich zu den bereits vorgestellten Methoden, wurden deshalb Reaktionssequenzen zur nachträglichen Acylierung von Tetramsäuren entwickelt (siehe Schema 11 und Schema 12). Dies ermöglicht zudem einen einfachen Zugang zu unterschiedlichen Seitenketten ohne die Gefahr von Nebenreaktionen in der Seitenkette während der Synthese.

1.5.1 Direkte 3-Acylierung

Aufbauend auf den Arbeiten von Kappler *et al.* zur Synthese von 3-Acyltetronsäuren (Lacton Analoge zu Tetramsäuren), entwickelte die Gruppe um

Jones eine Lewissäure-vermittelte Sequenz zur direkten Acylierung von 3H-Tetramsäuren **4a** mit Säurechloriden.^[85,86] In der Reaktion kommt es zur Bildung eines isolierbaren Bordifluorid-Komplexes **46**, welcher eine leichte Aufreinigung der normalerweise stark polaren 3-Acyltetramsäuren ermöglicht und eine ungewollte Komplexierung verhindert. Dieser Komplex kann nachträglich einfach mit Methanol unter Freisetzung der 3-Acyltetramsäure **5a** hydrolysiert werden.^[87,88] Mit der von Jones entwickelten Methode, war es erstmals möglich auch bis dato nur schwer zugängliche, ungesättigte 3-Acyltetramsäuren einfach darzustellen (siehe Schema 13).



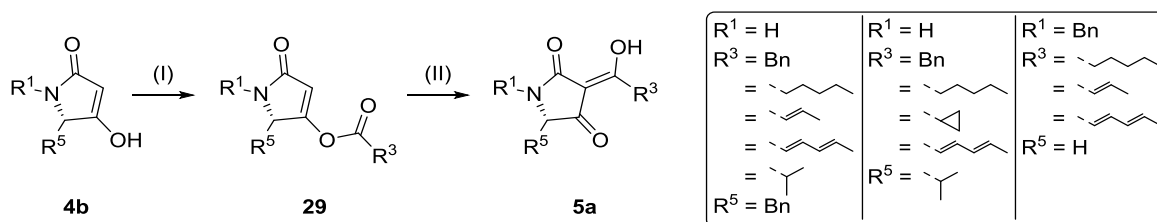
Schema 13: C-3 Acylierung von Tetramsäuren **4a** nach Jones *et al.*
 Reagenzien und Bedingungen: (I) ClOR^3 , $\text{BF}_3 \times \text{Et}_2\text{O}$, 80 °C; (II) MeOH, reflux.^[86,87]

Ebenso konnte in Totalsynthesen wie der des bereits beschriebenen Naturstoffes Tenuazonsäure (**7**) (siehe Schema 11) oder auch der Melophline (siehe Schema 12) die Verwendbarkeit dieser Methode gezeigt werden. Die Notwendigkeit von großen Überschüssen an Säurechloriden sowie die drastischen Reaktionsbedingungen (starke Lewissäuren und Rückfluss) limitieren diese Acylierungsmethode aber auf säure- und temperaturstabile Seitenketten und Funktionalitäten. Die Methode wird deshalb hauptsächlich für leicht zugängliche Seitenketten angewandt, da durch den notwendigen Überschuss an Säurechlorid es sich um keine ökonomische Darstellung von 3-Acyltetramsäuren handelt.

1.5.2 3-Acylierung via 4-O \rightarrow 3-C-Acylshift

Eine Alternative, zu der von Jones beschriebenen Methode, stellt die Umlagerung von 4-O-Acyltetramaten **29** in die entsprechenden 3-Acyltetramsäuren **5a**, dar.^[89,90] Dabei wird eine Säure mit dem C-4 Enolsauerstoff einer 3H-Tetramsäure **4b** verestert. Das kinetische Produkt, das 4-O-Acyltetramat **29**, wird anschließend

durch Basenzugabe in einer Fries-ähnlichen Verschiebung in das thermodynamische 3-Acyl-Produkt **5a** umgelagert.^[91] Dieser Syntheseweg besitzt auf Grund der milderer Reaktionsbedingungen eine weitaus höhere Toleranz an funktionellen Gruppen im Vergleich zu der beschriebenen direkten 3-Acylierung nach Jones.

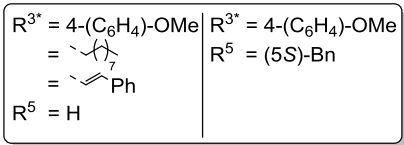


Schema 14: Darstellung von 3-Acyltetramsäuren **5a** via 4-O→3-C Acylshift.
 Reagenzien und Bedingungen: (I) DCC, DMAP, $R^3\text{COOH}$, CH_2Cl_2 , rt; (II) NEt_3 , CH_2Cl_2 , rt.^[89]

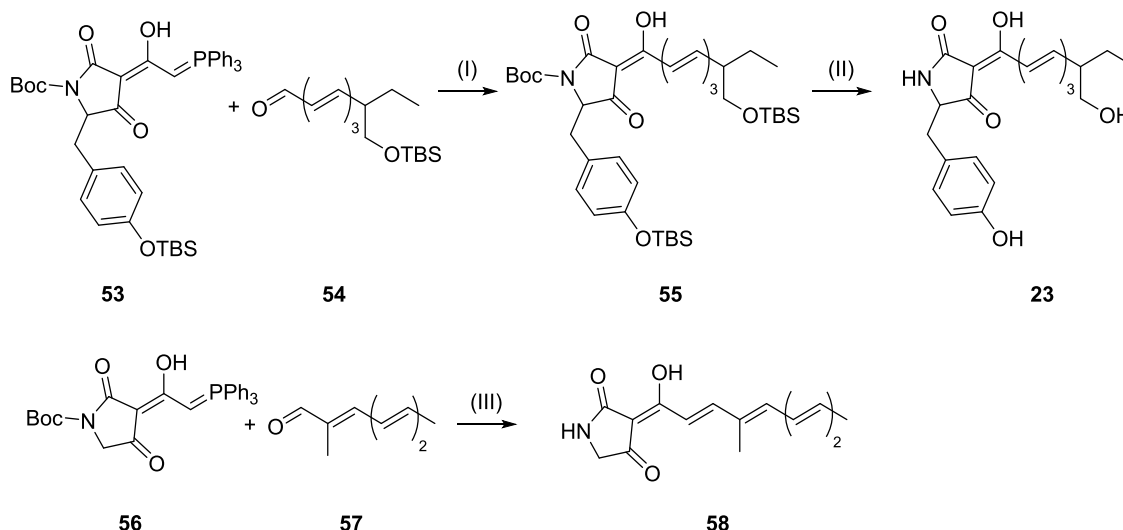
Mit den von Yoshii *et al.* vorgestellten Reaktionsbedingungen (siehe Schema 14) und später auch von Moloney *et al.* benutzten Methode, kommt es jedoch bei der Verwendung von α -verzweigten, oder sterisch anspruchsvollen Säuren, sowie konjugierten Seitenketten zu deutlich geringeren Ausbeuten oder gar der Bildung von Zersetzungsprodukten.^[92,93] Der Gruppe um Yoda *et al.* war es möglich diese Problematik durch die Zugabe von wasserfreiem CaCl_2 während der Umlagerung zu umgehen, was diese Reaktionssequenz in der Praxis allgemein anwendbar machte. Es wird vermutet, dass eine Komplexbildung der Ca^{2+} Ionen in der Reaktion durch das DMAP und die 3H-Tetramsäure **4a/4b** eine Acylierung in C-3-Position beschleunigt und somit die Verwendung von auch sterisch anspruchsvollen und α -verzweigten Seitenketten möglich macht.^[93,94] Yoda verwendete diese CaCl_2 moderierte 4-O-Acylumlagerung zur erstmaligen Darstellung von Penicillenol A₁ (**50**), und konnte außerdem auf diese Weise den Erhalt der Stereochemie während der Umlagerung zeigen. Ebenso gelang es der Gruppe um Yoda bei der Synthese von **50** eine verkürzte *one-pot* Variante mit direkter 4-O-Acylierung und gleichzeitiger Umlagerung zu etablieren und die Reaktionsführung somit weiter zu vereinfachen.



Reagenzien und Bedingungen: (I) *N*-Methylmorpholin, NEt₃, Et₂O, 0 °C; (II) (a) DMAP, CaCl₂, CH₂Cl₂, rt; (b) NaHMDS, MeI, THF, –40 °C; (c) HCl, MeOH, rt.^[93]



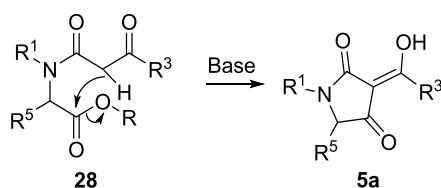
Reagenzien und Bedingungen: (I) THF, reflux, 16 h; (II) KO^tBu, R³*CHO, THF, reflux, 4-6 h.^[51,95]



Schema 17: Darstellung von Torrubiellon D (**23**) und Raveninsäure (**58**) via 3-Acylylidentetramsäuren. Reagenzien und Bedingungen: (I) KOtBu, THF, reflux, 20 min; (II) (a) TFA, CH₂Cl₂, rt, 1 h; (b) TFA, MeOH/H₂O, rt, 18 h; (III) (a) KOtBu, THF, reflux; (b) TFA, CH₂Cl₂, rt.^[51]

1.5.4 Darstellung von 3-Acyltetramsäuren per Lacey-Dieckmann Zyklisierung

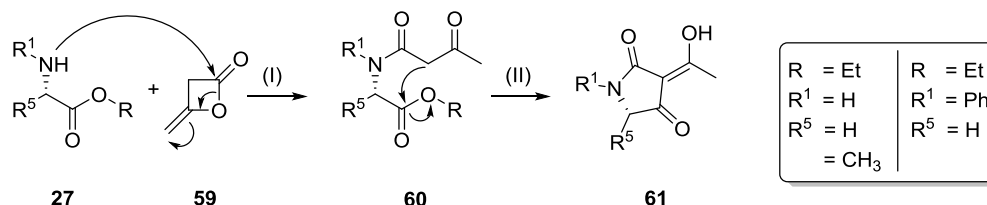
Neben der Darstellung von Tetramsäuren, gefolgt von ihrer 3-Acylierung, ist die zweite generelle Möglichkeit zur Darstellung von 3-Acyltetramsäuren, die bereits erwähnte Lacey-Dieckmann-Zyklisierung entsprechender *N*-(β -Ketoacyl)-aminosäureester **28**.



Schema 18: Mechanismus der basischen Kondensation von β -Ketoamiden **28** zur Darstellung von 3-Acyltetramsäuren **5**.

Diese stellt die vermutlich am häufigsten in der Naturstoffchemie verwendete Methode zum Aufbau von 3-Acyltetramsäuren **5** dar. Die nötigen Precursor, die β -Ketoamide **28**, werden hierzu meist aus Aminosäureesterderivaten **27** generiert. Die Darstellung des 3-Acyl-pyrrolidin-2,4-dions (**5a**) erfolgt in einer Basen induzierten Dieckmann-Kondensation. Sie erlaubt, ausgehend von *N*-(β -Ketoacyl)-aminosäureester **28** die direkte Synthese von 3-Acyltetramsäuren **5** ohne die Notwendigkeit einer nachträglichen Modifikation.

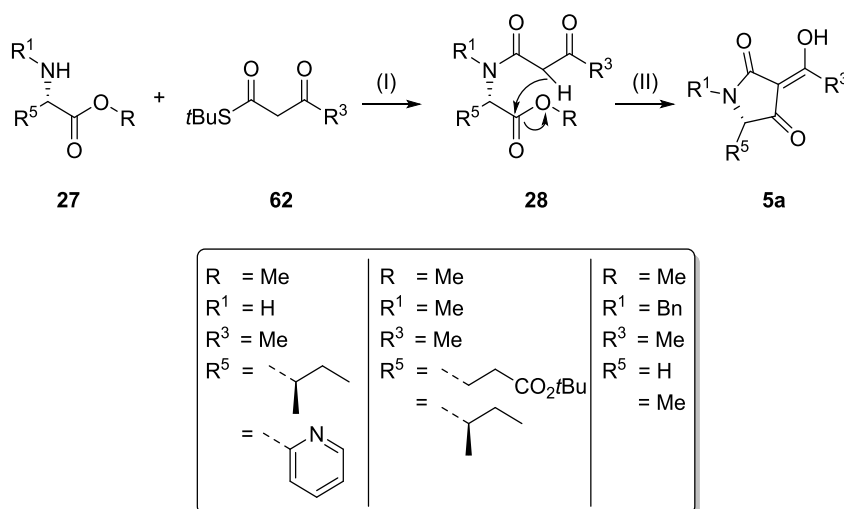
Dieser, von der Biosynthese von Tetramsäure abgeleitete Syntheseweg (siehe Kapitel 1.2), wurde erstmalig 1954 von Lacey zur Darstellung der 3-Acetyltetramsäuren **61**, einem Sonderfall der 3-Acyltetramsäuren, angewandt.^[96]



Schema 19: Darstellung von 3-Acetyltetramsäuren **61** nach Lacey.
Reagenzien und Bedingungen: (I) EtOH, rt, 1 h; (II) NaOMe, PhH, 3 h.^[96]

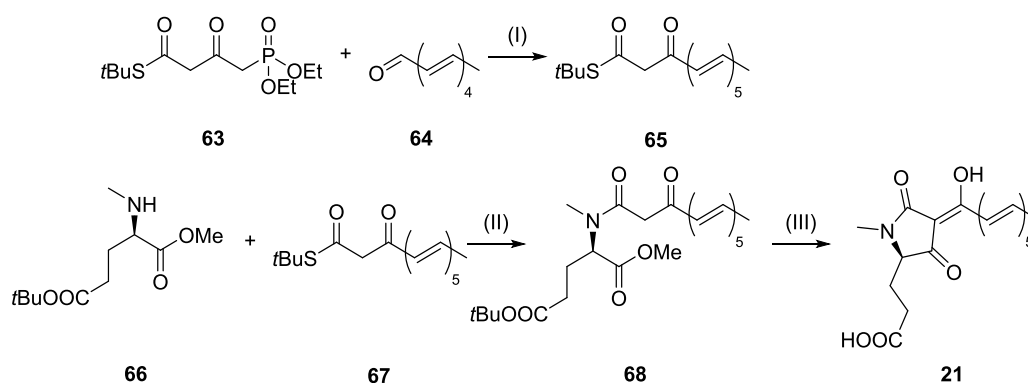
Wie in Schema 19 zu sehen, können durch die Umsetzung von Aminosäureestern **27** mit Diketen (**59**) ausschließlich *N*-Acetoacetamidaminosäureester **60** erhalten werden. Eine intramolekulare basische Esterkondensation, eine in diesem Kontext sogenannte Lacey-Dieckmann-Kondensation, führt anschließend zu den 3-Acetyltetramsäuren **61**.^[96,97] Da die Verwendung von Diketen (**59**) aber keine Variation der 3-Acylseitenkette erlaubte, waren weitere Modifikationen nötig um eine allgemein verwendbare Syntheseroute zur Darstellung von natürlichen und synthetischen Derivaten von 3-Acyltetramsäuren zu erreichen.

Die Gruppe um Ley *et al.* erweiterte die Methode zur Darstellung von **5** via Dieckmann-Kondensation zur heute hauptsächlich angewandten Variante, durch die Verwendung stabiler β -Ketothioester **62** zur Synthese verschiedener β -Ketoamide **28**. Diese erlauben sanftere Reaktionsbedingungen, verglichen zu den bis dahin bekannten Varianten, ausgehend von β -Ketoestern. Die Silbervermittelte Amidierung ermöglichte die Verwendung auch nur schwach nucleophiler Amine und chiraler Aminoverbindungen ohne nachweisbare Racemisierung.^[98] Aufbauend auf diesen und den Ergebnissen ihrer 3-Acyltetramsäuresynthesen entwickelten Ley und Woodward einen einfachen modularen Weg zur Darstellung unterschiedlichster 3-Acyltetramsäuren ausgehend von einfach darstellbaren Verbindungen.^[57] Die in Schema 20 gegebenen Beispiele zeigen nur einen Bruchteil der von Ley und Woodward untersuchten Substrate.



Schema 20: Allgemeine Darstellung von 3-Acyltetramisäuren **5a** via Dieckmann-Zyklisierung nach Ley *et al.*
 Reagenzien und Bedingungen: (I) AgO_2CCF_3 , THF, rt; (II) TBAF, THF, rt.

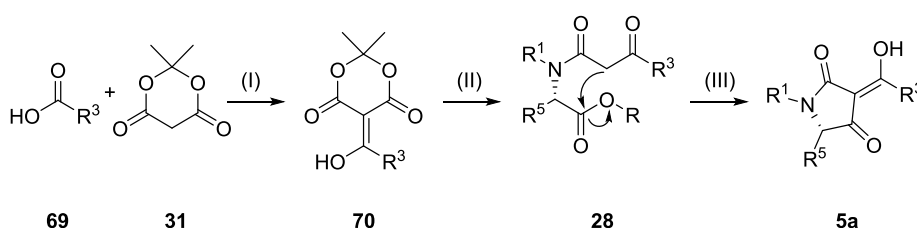
Ley etablierte zusätzlich die Verwendung von *tert*-Butyl-4-diethyl-phosphono-3-oxo-butanthioat (**63**) als modularen Baustein zur Darstellung von β -Ketothioestern. Dieses Thioesterphosphonat **63** erlaubt die Synthese sowohl konjugierter als auch gesättigter 3-Acyltetramisäuren. Eines der ersten Beispiele in der Anwendung der Lacey-Dieckmann Reaktion zur Synthese von Naturstoffen mit der Verwendung von **63**, ist die Totalsynthese der 3-Oligoenoyltetramisäure Fuligorubin A (**21**). Das mit Silbertrifluoracetat erhaltene β -Ketoamid **68** wurde in einer basen-induzierten Lacey-Dieckmann Reaktion mit nachfolgender globaler Entschützung in das Produkt **21** überführt (siehe Schema 21).^[99]



Schema 21: Darstellung von Fuligorubin A (**21**) via Lacey-Dieckmann-Zyklisierung.
 Reagenzien und Bedingungen: (I) NaH, THF, 0 °C, 25 min; (II) AgO_2CCF_3 , Na_2HPO_4 , rt, 3 h; (III) (a) KO^tBu , $n\text{BuOH}$, rt, 30 min; (b) HCOOH , rt, 1 h.^[99]

Zusätzlich zu der Verwendung des C4 Bausteins **63** von Ley, wurden weitere Möglichkeiten zur Darstellung von Dieckmann-fähigen β -Ketoamiden **28** identifiziert. Eine Möglichkeit zur Synthese von **28** stellt, die zum damaligen

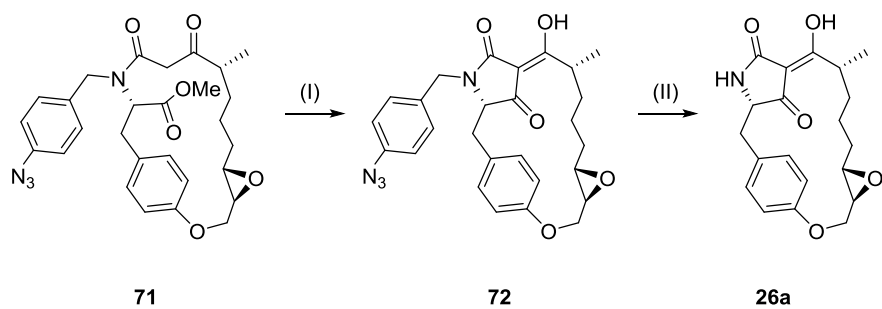
Zeitpunkt bereits für die Darstellung von β -Ketoestern verwendete, Meldrumsäure (**31**) dar. In der Reaktion kommt es im ersten Schritt zur Acylierung von **31** mit aktivierten Säuren **69**. Das gebildete Acyl-Meldrumsäureaddukt **70** wird anschließend in einer thermolytischen Aminolyse mit Aminosäureesterderivaten **27** in ein β -Ketoamid **28** überführt.^[100,101] Die Synthese von **28** (siehe Schema 22) über diese Variante findet aber trotz ihrer Einfachheit, auf Grund der Instabilität des Addukts **70** eher selten Anwendung in Naturstoffsynthesen.



Schema 22: Allgemeine Darstellung von β -Ketoamiden **28** via Meldrumsäure (**31**).

Reagenzien und Bedingungen: (I) DMAP, EDCI, rt, 16 h; (II) **27**, Dioxan, reflux, 2,5 h; (III) NaOMe, MeOH, rt.

Obwohl die Lacey-Dieckmann Zyklisierung, wie bereits erwähnt, die häufigste angewandte Methode zur Darstellung von 3-Acyltetramsäuren ist, birgt sie ein gewisses Risiko. Auf Grund der Notwendigkeit von starken Alkoholat-Basen wie NaOMe während der Dieckmann-Kondensation kommt es je nach Reaktionsführung zur teilweisen Epimerisierung an der C-5 Position des Pyrrolidin-2,4-dions.^[102] Die Verwendung von KO^tBu oder TBAF als Zyklisierungsbase minimiert diese Erscheinung aber erheblich.^[57,99] In Folge der Tolerierung verschiedener funktioneller Gruppen und der Einfachheit der Durchführung konnten auf diese Weise neben den bereits angeführten Beispielen, eine Vielzahl von natürlichen und nicht natürlichen 3-Acyltetramsäuren **5** mit verschiedensten Komplexitäten dargestellt werden.^[103–106] So war es beispielsweise möglich sowohl makrozyklische 3-Acyltetramsäuren wie das Macrocidin A (**26a**) (siehe Schema 23) wie auch konjugiert ungesättigte 3-Acyltetramsäuren wie das bereits beschriebene Fuligorubin A (**21**) (siehe Schema 21) oder β -Lipomycin (**15**) stereoselektiv zu synthetisieren.^[42,57,104]



Schema 23: Darstellung von Macrocidin A (**26a**) via Lacey-Dieckmann-Zyklisierung.
 Reagenzien und Bedingungen: (I) KO^tBu, *t*BuOH, THF, rt, 40 min; (II) (a) H₂, Pd/C, MeOH, THF, rt, 2 h; (b) DDQ, H₂O, THF, rt, 30 min

2 Zielsetzung

In der Wirkstoffentwicklung werden häufig Methoden zur Modifizierung von Wirkstoffen gesucht, um eine selektive Adressierung eines Targets zu ermöglichen. Saccharide finden dabei bereits als *Targeting and Drug Delivery System* sowohl in der Impfstoffentwicklung, als auch in der Krebstherapie Anwendung. Darin haben Mono- und Oligosaccharide meist den Sinn als eine leicht abspaltbare spezifische Modifikation eines Wirkstoffes zu fungieren.^[107,108] Die enthaltenen Saccharide dienen als Marker für endogene Rezeptoren auf Zelloberflächen, die sogenannten Lektine.^[109] Durch diese ist es möglich die gezielte Adressierung von Targets zu erreichen um als Folge daraus die therapeutische Dosis zu senken oder Nebenwirkungen im Allgemeinen zu verringern.^[110] Des weiteren erhöhen Saccharide die Wasserlöslichkeit der oftmals lipophilen Wirkstoffe oder ändern, wie bereits an pharmazeutisch verwendeten Beispielen zu sehen ist, die Konformation eines Substrats und ermöglichen so eine bessere Aufnahme in den Organismus.^[111]

Basierend auf diesen Überlegungen war das Ziel dieser Arbeit die Erstsynthese von Saccharid-gekuppelten natürlichen 3-Acyltetramsäuren. Neben der Synthese von noch nicht untersuchten Verbindungsklassen sollten, zu den bereits während den Isolationen durchgeführten Bioaktivitätstests, weitere Untersuchungen der biologischen Wirkungen der synthetisch erhaltenen Verbindungen gemacht werden. Dabei sollte primär die Toxizität der einzelnen Naturstoffe gegenüber üblichen Krebszelllinien getestet werden. Neben diesen sollte auf Grund der oftmals beobachteten antibiotischen Wirkung von 3-Acyltetramsäuren die Wirkung dieser Verbindungen gegenüber unterschiedlichen Bakterienstämmen getestet werden. Um ein besseres Verständnis der Struktur-Wirkungsbeziehung zu erhalten, sollten ebenfalls etwaige Vorstufen oder Derivate der Naturstoffe untersucht werden.

Aufbauend auf den bereits durchgeführten Arbeiten zur Synthese von 3-Acyltetramsäuren in der Arbeitsgruppe Schobert, war es das Ziel natürliche glykosylierte 3-Acyltetramsäuren totalsynthetisch darzustellen. Dabei sollten zusätzlich zu den bereits literaturbekannten Synthesen der glykosylierten Verbindungen Aflastatin A,^[112] Epicoccamid D^[113], α -Lipomycin (**16**)^[114] und

Streptolydigin (**20**) ^[115] neuartig glykosylierte Derivate von 3-Acyltetramsäuren dargestellt werden.

Die Arbeit unterteilte sich in zwei Themen. Im ersten Projekt sollten *N*-glykosylierte Naturstoffen der Gruppe der Aurantoside synthetisch untersucht werden. Hierzu war es das Ziel einen ersten Zugang zu dieser außergewöhnlichen Gruppe an sowohl halogenierten als auch konjugiert ungesättigten Naturstoffen zu erarbeiten. Im zweiten Projekt sollten *O*-diglykosidische 3-Acyltetramsäuren der Gruppe der Ancorinoside erarbeitet werden. Hierbei sollte ebenfalls, aufbauend auf den bestehenden Arbeiten, eine möglichst flexible Syntheseroute der Totalsynthese verwirklicht werden. Die Flexibilität der erarbeiteten Synthesestrategie sollte mit der totalsynthetischen Darstellung der Derivate Ancorinosid A (**74**) und Ancorinosid D (**75**), belegt werden. Von besonderem Interesse war dabei die Synthese des Disaccharids und die Bestätigung der Stereochemie an der C-5 Position des Pyrrolidin-2,4-dions, basierend auf einer nicht proteinogenen Aminosäure.

3 Synopsis

Die vorliegende kumulative Dissertation umfasst drei wissenschaftliche Publikationen, welche in Kapitel 6 abgedruckt sind. Diese Arbeit handelt von der Darstellung natürlicher 3-Acyltetramsäuren, mit unterschiedlich glykosyliertem Mono- beziehungsweise Disaccharidrest, via Lacey-Dieckmann Zyklisierung. Die Dissertation unterteilt sich dabei in zwei Projekte.

Im ersten Projekt sollte die Klasse der Aurantoside erstmals mit der Totalsynthese eines der einfachsten Vertreter, dem Aurantosid G (**73**) (siehe Abbildung 10), zugänglich gemacht werden. Die insgesamt elf Derivate dieser Gruppe an halogenierten und *N*-glykosylierten 3-Polyenoyltetramsäuren sind mit Streptolydigin (**20**) und zusammen mit den zu Aurantosiden analogen Rubrosiden (siehe Abbildung 10 Rubrosid H (**74**)), die einzigen bis dato bekannten, natürlichen *N*-glykosylierten 3-Oligoenoyltetramsäuren.

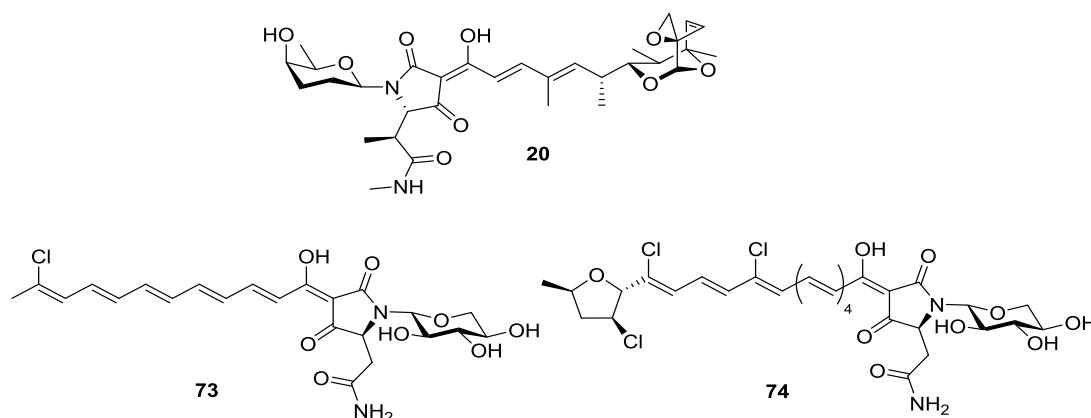


Abbildung 10: Struktur der *N*-glykosylierten 3-Oligoenoyltetramsäuren Streptolydigin (**20**), Aurantosid G (**73**) und Rubrosid H (**74**).

Die in der Synthese von **73** gewonnen Erkenntnisse zur Darstellung von 3-Acyltetramsäuren wurden im zweiten Teilprojekt auf die Synthese von weiteren glykosylierten Pyrrolidin-2,4-dionen angewandt. Das zweite Teilprojekt bestand aus der Erarbeitung einer ebenfalls flexiblen Synthese von *O*-diglykosidischen Tetramsäuren, zur Darstellung möglichst verschiedener Vertreter der Klasse der Ancorinoside. Dabei sollten die beiden Derivate Ancorinosid A (**75**) und D (**76**) erstmals synthetisiert werden. Diese weisen eine gleiche Saccharidstruktur, aber eine unterschiedliche 3-Acylkette (C22 gegen C24) mit bzw. ohne zusätzlicher *cis* Doppelbindung auf. Beide Derivate (siehe Abbildung 11) wurden auf Grund ihrer

strukturellen Unterschiede gewählt, um die Flexibilität und Anwendbarkeit der erarbeiteten Synthesestrategie zu zeigen.

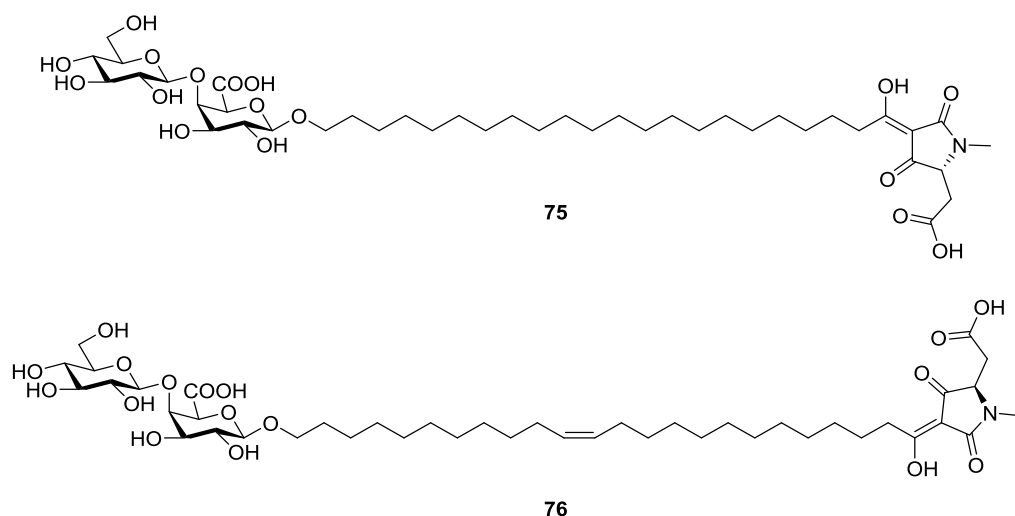


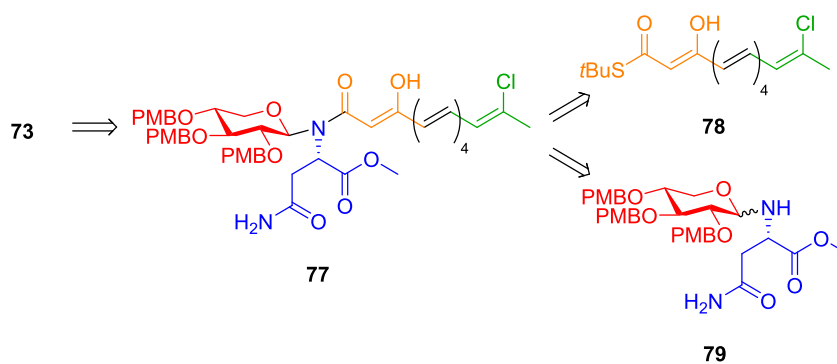
Abbildung 11: Strukturen der beiden O-diglykosidischen 3-Acyltetramsäuren Ancorinosid A (**75**) und Ancorinosid D (**76**).

Für die jeweiligen Synthesen ist die in Kapitel 1.5.4 erläuterte Lacey-Dieckmann Zyklisierung als zentrale Schlüsselreaktion zum Aufbau des Pyrrolidin-2,4-dion Rings herangezogen worden. Dies erlaubte einen möglichst späten Einbau des polaren Heterozyklus und damit die Verwendung leichter handhabbarer Intermediate.

3.1 Synthese *N*-glykosylierter 3-Acyltetramsäuren

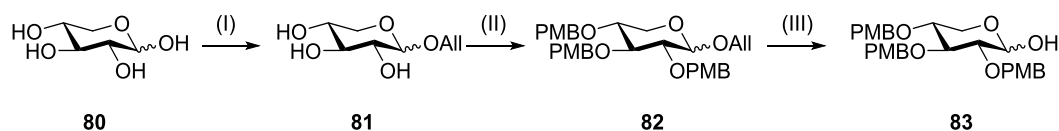
3.1.1 Totalsynthese von Aurantosid G, einer *N*- β -glykosylierten 3-Oligoenoyltetramsäure aus dem marinen Schwamm *Theonella swinhoei*

Erster Teil dieser Arbeit ist die Synthese von Aurantosid G (**73**) als Erstsynthese einer konjugiert ungesättigten *N*-glykosylierten 3-Acyltetramsäure mit der formalen Summenformel $C_n(H_2O)_m$ des enthaltenen Monosaccharids.^[116,117] Die Synthese von Aurantosid G basiert auf den zwei Kernbausteinen **78** und **79**, welche im Schema 24 dargestellt sind.



Schema 24: Retrosynthetische Betrachtung von Aurantosid G (**73**).

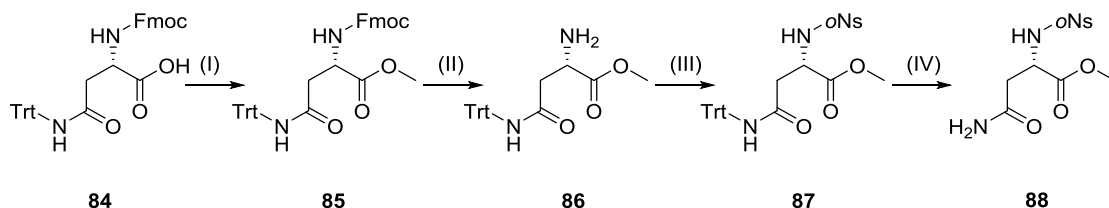
Zum Aufbau des *N*-glykosylierten Motivs, wurde das Schlüsselfragment **79** aus dem 2,3,4-Tri-*O*-(*para*-methoxybenzyl)-D-xylopyranosid (**83**) (in Schema 24 rot dargestellt) und dem *N*-nosylierten Methyl L-Asparaginat (**88**) (in Schema 24 blau dargestellt) synthetisiert. In der Synthese des Saccharidfragments **83** wurde, durch vorangehende Experimente, die Notwendigkeit einer elektronenreichen Schutzgruppe der OH Gruppen insbesondere an der Position C-2 des D-Xylosylrestes herausgearbeitet. Erst durch die Verwendung der *para*-Methoxybenzyl-Gruppe konnte die für die spätere Aminolyse nötige Elektronendichte am anomeren Stickstoff in **79** erhalten werden. Das Xylosederivat **83** wurde in drei Stufen mit einer Gesamtausbeute von 58% nach literaturbekannten Methoden aufgebaut. Dabei wurde D-Xylose (**80**) im ersten Schritt anomer allyliert, anschließend die Hydroxyfunktionalitäten PMB verethert und im letzten Schritt die anomere Allylgruppe wieder entfernt (siehe Schema 25).



Schema 25: Synthese des Saccharidfragments **83**.

Reagenzien und Bedingungen: (I) AlIOH , $\text{BF}_3 \times \text{Et}_2\text{O}$, reflux, 16 h, 77%; (II) PMBCl , NaH , DMF , $0^\circ\text{C} \rightarrow \text{rt}$, 72 h, 86%; (III) (a) KOtBu , DMF , 70°C , 2 h; (b) HCl , AcMe , reflux, 1 h, 87%.

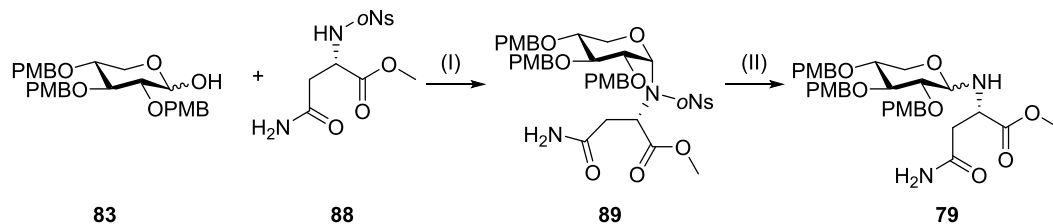
Das in **79** ebenfalls enthaltene L-Asparagin (in Schema 24 blau dargestellt) wurde nach Schutzgruppenmodifikation und *ortho*-Nosyl Kupplung in 80% über vier Stufen aus kommerziellem Fmoc-L-Asn(Trt)-OH (**84**) dargestellt (siehe Schema 26). Diese Nosyl-Aktivierung war nötig um eine ausreichende Acidität der N-H Bindung für die geplante *N*-Glykosylierung unter Fukuyama-Mitsunobu Bedingungen zu erhalten. Für die Darstellung von **73** wurde die in **84** enthaltene Amidschutzgruppe Trityl bereits frühzeitig an dieser Stelle der Totalsynthese entfernt. Versuche der Freisetzung des Amids in späteren Stufen der Synthese, vor allem mit enthaltener konjugierter Seitenkette, führten zu einer Zersetzung der Produkte.



Schema 26: Synthese des Aminosäurebausteins **88**.

Reagenzien und Bedingungen: (I) K_2CO_3 , MeI , DMF , $0^\circ\text{C} \rightarrow \text{rt}$, 1 h, 99%; (b) Piperidin, DMF , rt, 1 h, 99%; oNsCl , NEt_3 , CH_2Cl_2 , $0^\circ\text{C} \rightarrow \text{rt}$, 16 h, 92%; (IV) TFA , CH_2Cl_2 , $0^\circ\text{C} \rightarrow \text{rt}$, 1 h, 99%.

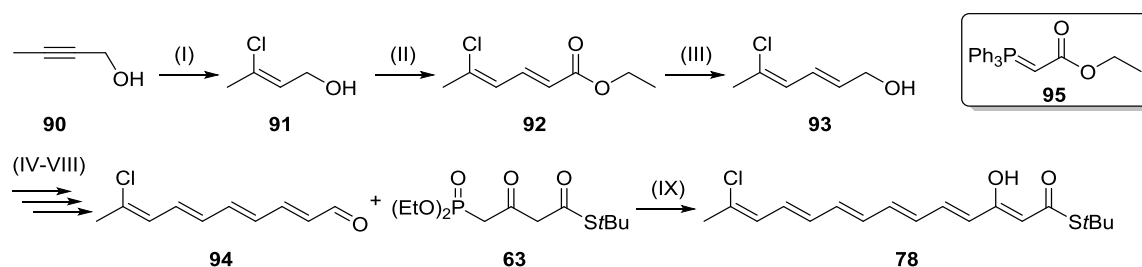
In der Kondensationsreaktion dieser *ortho*-Nosyl aktivierten Aminosäure **88** mit dem Saccharid **83** konnte nur das thermodynamisch stabilere α -Anomer isoliert werden. Eine Abspaltung der Nosyl-Gruppe via nucleophiler aromatischer Substitution durch Thiophenol mit intermediär gebildetem Meisenheimer-Komplex, lieferte schließlich den Kernbaustein **79** als *N*-glykosyliertes Anomerengemisch.



Schema 27: Darstellung des Schlüsselfragments **79**.

Reagenzien und Bedingungen: (I) PPh_3 , DIAD , THF , $-78^\circ\text{C} \rightarrow \text{rt}$, 16 h, 69%; (II) DIPEA , PhSH , DMF , rt, 4 h, 77%.

Das β -Ketothioestermotiv im zweiten Schlüsselfragment **78** wurde, nach der in Kapitel 1.5.4 beschriebenen Methode von Ley, über eine HWE-Reaktion mit Phosphonat **63** (in Schema 24 orange dargestellt) erzeugt. Ausgehend von But-2-in-1-ol (**90**) wurde der chlorierte allylische Alkohol **91** (in Schema 24 grün dargestellt) in einer OH-Gruppen dirigierenden anti-Hydroaluminierung erzeugt und sukzessiv um weitere konjugierte Doppelbindungen verlängert. Der labile Alkohol **91** wurden in einer Eintopfreaktion mit MnO_2 zum entsprechenden Aldehyd oxidiert und dieser mit dem ebenfalls anwesenden Ylid **95** direkt zu dem stabilen konjugierten Ester **92** olefiniert. Nach Reduktion dieses Esters zum allylischen Alkohol **93** wurde dieser nach derselben Methode wiederum verlängert. Die jeweils repetitiven Schritte der Oxidationen mit in situ Olefinierungen und anschließenden Reduktionen zu den allylischen Alkoholen sowie die abschließende Oxidation zum Aldehyd **94** (Schritte IV-VIII) sind in Schema 28 zusammengefasst dargestellt.

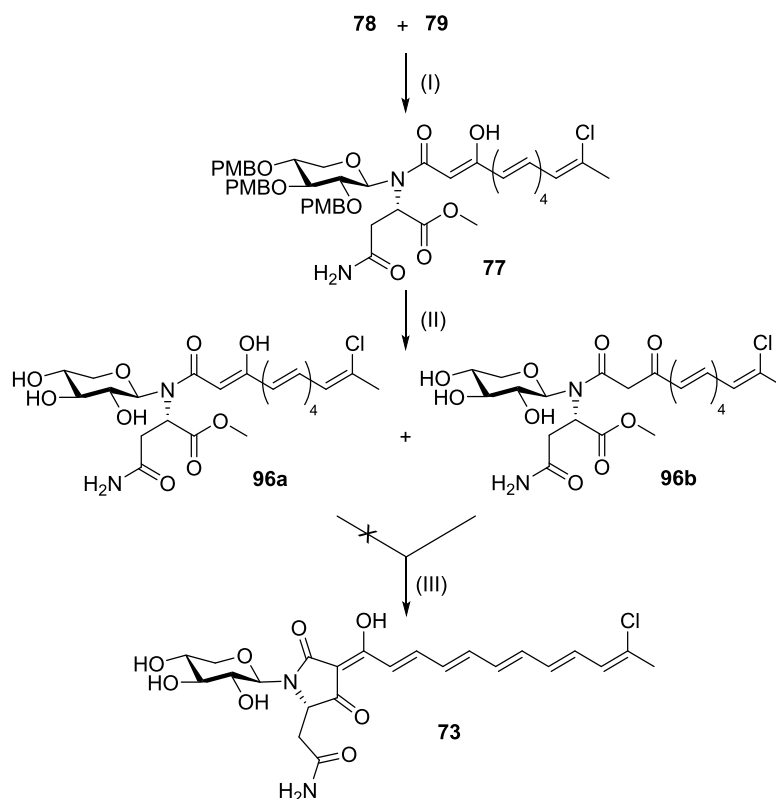


Schema 28: Darstellung des konjugierten Thioesters **78**.

Reagenzien und Bedingungen: (I) (a) Red-Al, THF, 0 °C \rightarrow rt, 16 h; (b) NCS, THF, -78 °C \rightarrow 0 °C, 3 h, 82%; (II) MnO_2 , **95**, CH_2Cl_2 , reflux, 16 h, 63 %; (III) DIBAL-H, CH_2Cl_2 , -78 °C, 1 h, 75%; (IV) MnO_2 , **95**, CH_2Cl_2 , reflux, 16 h, 77%; (V) DIBAL-H, CH_2Cl_2 , -78 °C, 1 h, 94 %; (VI) MnO_2 , **95**, CH_2Cl_2 , reflux, 16 h, 60%; (VII) DIBAL-H, CH_2Cl_2 , -78 °C, 77%; (VIII) MnO_2 , CH_2Cl_2 , rt, 3 h; (IX) NaH, THF, 0 °C, 3 h, 84 % (über 2 Stufen).

Der β -Ketothioester **78** wurde in einer HWE-Reaktion aus dem konjugierten ungesättigten Aldehyd **94** und dem Thioesterphosphonat **63** *E*-selektiv synthetisiert.

Einem allgemeinen Schema zur Synthese von Amiden nach Ley und Woodward folgend (vgl. Schema 20) wurde der Thioester **78** mit dem *N*-Glykosid **79** kondensiert. Die verwendete PMB-Schutzgruppe führte hierbei auf Grund ihres sterischen Anspruchs zur Bildung des β -Anomers **77**. Nach Abspaltung der PMB-Schutzgruppen wurde der resultierende *N*-(β -Ketoacyl)aminoester **96b** unter basischen Bedingungen in einer Lacey-Dieckmann-Reaktion zur Zieltetransäure, dem natürlichen Aurantosid G (**73**), zyklisiert (siehe Schema 29).

Schema 29: Darstellung von Aurantosid G (**73**).

Reagenzien und Bedingungen: (I) AgO_2CCF_3 , MS 4 Å, THF, 0 °C, 3 h, 49%; (II), TFA, PhOMe, CH_2Cl_2 , 0 °C, 15 min, 71%; (III) NaOMe, MeOH, rt, 10 min, quant..

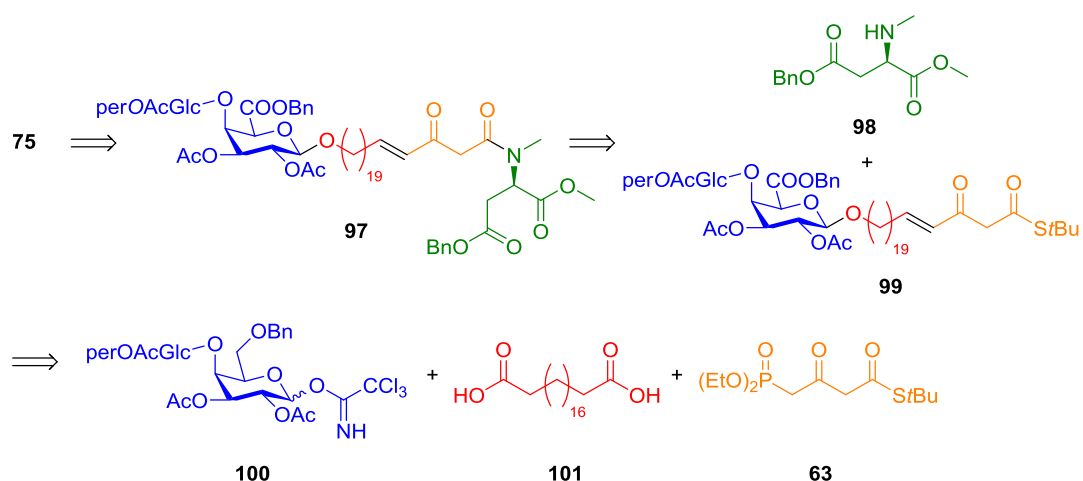
Unerwarteterweise wurden unter diesen Bedingungen nur das β-Ketoamid **96b** und nicht das β-Enolamid **96a**, welche trennbar nebeneinander vorlagen, zur Reaktion gebracht. Die Deprotonierung des Enols **96a** scheint erschwert möglich zu sein womit die resultierende Bildung des thermodynamisch stabilen Pyrrolidin-2,4-dion-Rings nur verlangsamt abläuft. Über die beschriebene Synthese von **73** wurde erstmal ein Zugang zur Gruppe dieser halogenierten und *N*-glykosylierten 3-Oligoenoyltetramsäuren eröffnet. Biologische Untersuchungen der antibiotischen und zytotoxischen Aktivitäten konnten aber aufgrund der hohen Sensibilität der konjugierten Kette sowohl gegenüber Licht als auch Oxidationsreaktionen nicht durchgeführt werden.

3.2 Synthese O-glykosylierter 3-Acyltetramsäuren

Aufbauend auf den gewonnenen Erkenntnissen der Saccharidsynthese und der Chemie der 3-Acyltetramsäuren, insbesondere im Umfeld der Lacey-Dieckmann Zyklisierung, war es das nächste Ziel neben den *N*-glykosylierten, auch die Gruppe der O-glykosidischen 3-Acyltetramsäuren zu untersuchen. Hierzu sollte eine möglichst allgemein anwendbare Methode zu deren Synthese erarbeitet werden.

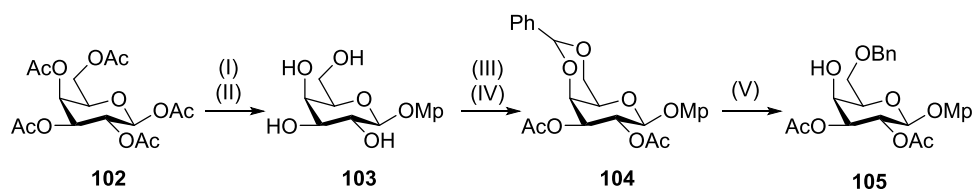
3.2.1 Erste Totalsynthese der O-diglykosidischen Tetramsäure Ancorinosid A

Ancorinosid A (**75**), eine diglykosidische 3-Acyltetramsäure, sollte analog zu Aurantiosid G (**73**) via Lacey-Dieckmann-Zyklisierung und unter Verwendung von Ley's Thioester **63** (siehe Schema 28) aufgebaut werden. Dabei lag das Hauptaugenmerk auf der Darstellung der diglykosidischen Galacturonsäure (in Schema 30 blau dargestellt). Zusätzlich sollte die in der Publikation der Isolierung zugewiesene Stereochemie der C-5 Position von **75** bestätigt werden und neben den schon veröffentlichten Bio-Aktivitätstests weitere Zytotoxizitäts- und Antibiotiktests durchgeführt werden. Analog zu **73** wurde **75** aus mehreren Bausteinen aufgebaut. Dabei war Verbindung **99** mit dem β -Ketothioester und dem vollständig geschützten Uronat das zentrale Intermediat.



Schema 30: Retrosynthetische Betrachtung von Ancorinosid A (**75**).

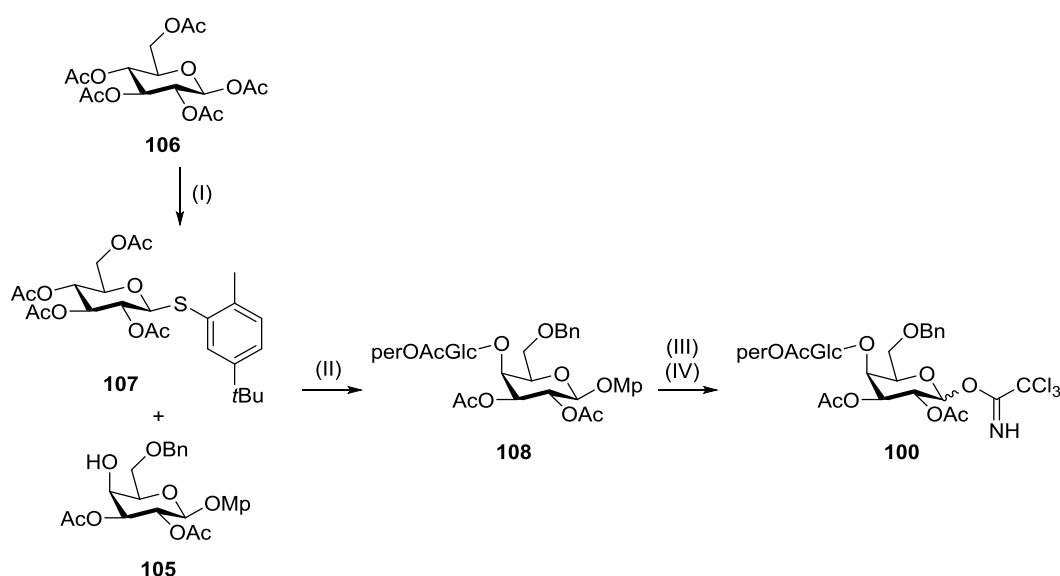
Zunächst wurde das Disaccharid **100** synthetisiert. Hierfür konnte nach Schutzgruppenmodifikation des 1,2,3,4,6-Penta-O-acetyl- β -D-Galactopyranosids (**102**) in fünf Stufen das orthogonal geschützte und an Position C-4 freie Saccharid **105** in 60% Ausbeute erhalten werden.



Schema 31. Synthese des Galactoseakzeptors **105**.

Reagenzien und Bedingungen: (I) MgOH , NEt_3 , $\text{BF}_3 \times \text{Et}_2\text{O}$, CH_2Cl_2 , $0^\circ\text{C} \rightarrow \text{rt}$, 16 h, 98%; (II) NaOMe , MeOH , rt , 15 min, 96%; (III) PhCH(OMe)_2 , $p\text{TosOH} \times \text{H}_2\text{O}$, MeCN , rt , 1 h, 91%; (IV) Pyridin , Ac_2O , rt , 16 h, 91%; (V) Et_3SiH , TFA , CH_2Cl_2 , $0^\circ\text{C} \rightarrow \text{rt}$, 77%.

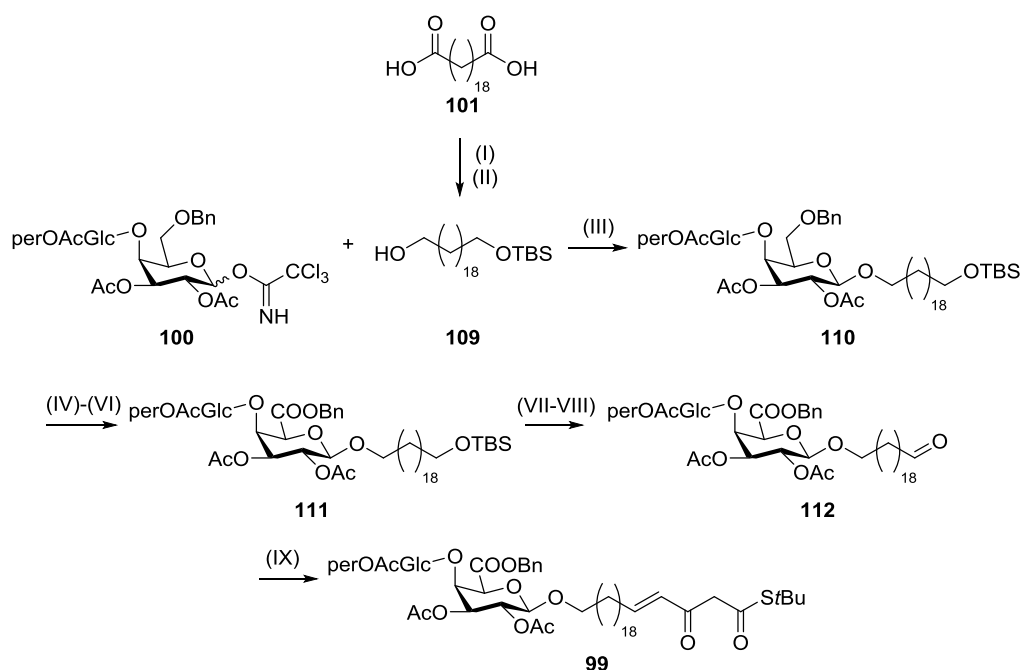
Akzeptor **105** wurde β -selektiv mit dem (2-Methyl-5-*tert*-butyl-phenyl)-2,3,4,6-tetra-O-acetyl-1-S- β -D-glucopyranosid (**107**) unter Bildung des Disaccharids **108** glykosyliert. **107** wurde in nur einer Stufe, analog zur Anomeren-Kupplung der peracetylierten β -D-Galactose **102** (vgl. Schema 31) aus der peracetylierten β -D-Glucose **106** und 2-Methyl-5-*tert*-butyl-thiophenol synthetisiert. Die detaillierte Durchführung zur Darstellung des literaturbekannten Galactosylakzeptors **105** und des ebenfalls literaturbekannten Glucosyldonors **107** sind in Kapitel 6.2 zu sehen.



Schema 32: Darstellung des diglycosidischen Imidats **100**.

Reagenzien und Bedingungen: (I) 2-Me-5-*t*Bu-Thiophenol, $\text{BF}_3 \times \text{Et}_2\text{O}$, CH_2Cl_2 , $0^\circ\text{C} \rightarrow \text{rt}$, 16 h, 92% (II) NIS , TfOH , $\text{MS } 4 \text{ \AA}$, CH_2Cl_2 , $-78^\circ\text{C} \rightarrow 0^\circ\text{C}$, 3 h, 82%; (III) CAN , AcMe , H_2O , rt , 30 min, 73%; (IV) K_2CO_3 , Cl_3CCN , CH_2Cl_2 , rt , 16 h, 77%.

Das vollgeschützte Dissacharid **108** wurde anschließend anomer entschützt und in das Imidat **100** überführt. Die Oxidation zur Uronsäuregruppe in der Galactose-Einheit wurde auf Grund der bekannt schlechten Ausbeuten und β -Selektivitäten in Glykosylierungsreaktionen mit Uronsäuren und Uronaten erst nachträglich durchgeführt. Dagegen erlaubte die Kupplung des Saccharids **100** unter Schmidt-Bedingungen eine β -selektive Glykosylierung des Spacers **109**. Dieser Spacer konnte durch die Reduktion von Eicosandisäure (**101**) zum Eicosandiol mit anschließender Monosilylierung einfach synthetisiert werden. Das nun Spacergekuppelte Saccharid **110** wurde, nach Freisetzung des benzyl-geschützten primären Alkohols, zur Galacturonsäure oxidiert und zum Benzyluronat **111** verestert. Dieses Saccharid **111** wurde nun essigsauer desilyliert und der erhaltene primäre Alkohol anschließend zum Aldehyd **112** oxidiert. Analog zur Synthese von Aurantiosid G (**73**) (siehe Schema 28) war es damit möglich das β -Ketothioestermotiv in **99** mittels HWE-Olefinierung von **63** mit **112** (siehe Schema 21 bzw. Schema 28) zu generieren.

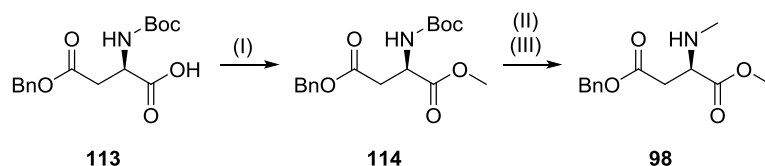


Schema 33: Darstellung des Schlüsselbausteins **99**.

Reagenzien und Bedingungen: (I) LiAlH_4 , THF, $0^\circ\text{C} \rightarrow \text{reflux}$, 16 h, 87%; (II) TBSCl, Imidazol, CH_2Cl_2 , reflux, 16 h, 66%; (III) MS 4 Å, TMSOTf, CH_2Cl_2 , $-40^\circ\text{C} \rightarrow \text{rt}$, 16 h, 73%; (IV) Pd/C, H_2 , EtOAc, rt, 3,5 h, 99%; (V) TEMPO, BAIB, CH_2Cl_2 , H_2O , rt, 2 h, 97%; (VI) K_2CO_3 , BnBr, DMF, $0^\circ\text{C} \rightarrow \text{rt}$, 3 h, 93%; (VII) AcOH, THF, H_2O , rt, 2,5 h, 92%; (VIII) DMP, rt, 2 h, 79%; (IX) (a) NaH, **63**, THF, 0°C , 1 h; (b) **99**, THF, rt, 16 h, 45%.

Amidierung dieses Thioesters **99** mit dem *N*-methylierten D-Aspartat **98** (siehe Schema 34) ergab schließlich den geschützten *N*-(β -Ketoacyl)aminosäureester

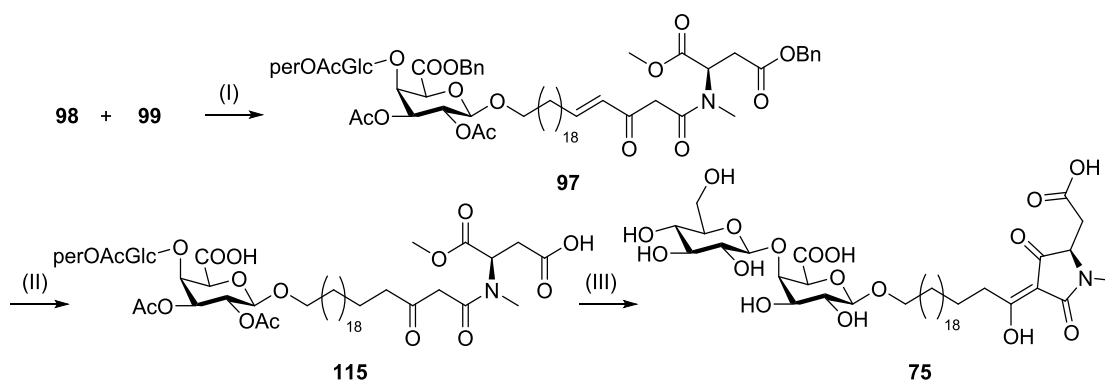
97. Das Aspartat **98** konnte durch Methylierung, *N*-Methylierung und Boc-Abspaltung in drei kurzen Schritten aus dem erhältlichen **113** synthetisiert werden.



Schema 34: Synthese des Aminosäurebausteins **98**.

Reagenzien und Bedingungen: (I) K_2CO_3 , MeI, DMF, $0^\circ\text{C} \rightarrow \text{rt}$, 1 h, 90%; (II) NaH, MeI, DMF, $0^\circ\text{C} \rightarrow \text{rt}$, 1,5 h, (III) TFA, CH_2Cl_2 , $0^\circ\text{C} \rightarrow \text{rt}$, 2 h, 68%.

Eine Freisetzung der beiden Säuregruppen mit simultaner Hydrierung der, in der HWE-Reaktion entstanden, Doppelbindung ermöglichte die Generierung von **115**, der direkten Vorstufe von **75**. Die abschließende Dieckmann-Zyklisierung mit gleichzeitiger Deacetylierung lieferte Ancorinosid A (**75**) in nur einem Schritt.



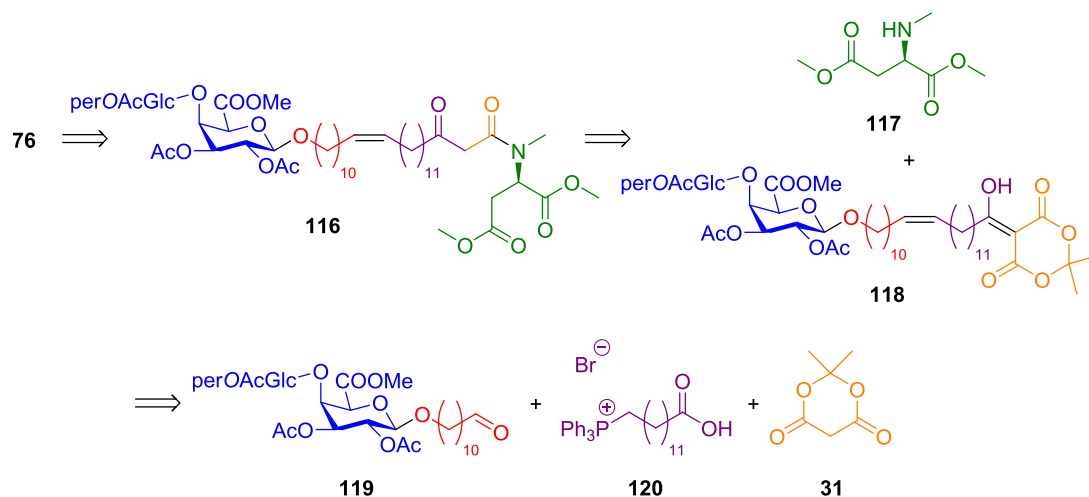
Schema 35: Darstellung von Ancorinosid A (**75**) via Lacey-Dieckmann-Zyklisierung.

Reagenzien und Bedingungen: (I) AgO_2CCF_3 , NEt_3 , THF, 0°C , 3 h, 63%; (II) Pd/C, H_2 , EtOAc, 8 h, 84%; (III) NaOMe, MeOH, rt, 1 h, 48 %.

Mit dieser Erstsynthese von **75** konnte trotz Abweichung des spezifischen Drehwertes des Syntheseprodukts von dem des natürlichen Isolats die publizierte Konfiguration des Heterozyklus via Zirkulardichroismus bestätigt werden. Durchgeführte MTT-Tests zur Bestimmung der Toxizität gegenüber 518A2 Melanomzellen zeigten mit einer Konzentration von mehr als $50\ \mu\text{M}$ keine Aktivität von **75**.

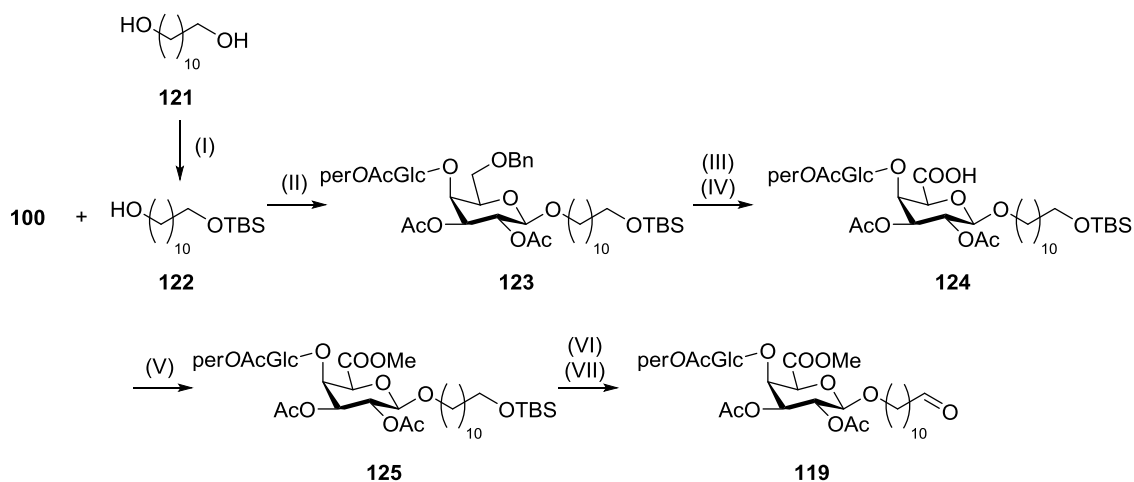
3.2.2 Eine synthetische Route zum MT1-MMP Inhibitor Ancorinosid D

Die Flexibilität der für Ancorinosid A (**75**) ausgearbeiteten Syntheseroute (siehe Kapitel 3.2.1) sollte nun mit der Synthese von Ancorinosid D (**76**) gezeigt werden. Die *cis*-Doppelbindung in **76** erforderte jedoch eine unterschiedliche Herangehensweise. Die Verwendung von Ley's Phosphonat **63** zur Einbringung eines Thioesters via HWE-Olefinierung würde eine zweite nicht separat adressierbare Doppelbindung erzeugen. Deshalb wurde der Aufbau des β -Ketoamidmotivs nach Pak *et al.* über ein Meldrumsäureaddukt gewählt (allgemeine Sequenz siehe Schema 22).^[100]



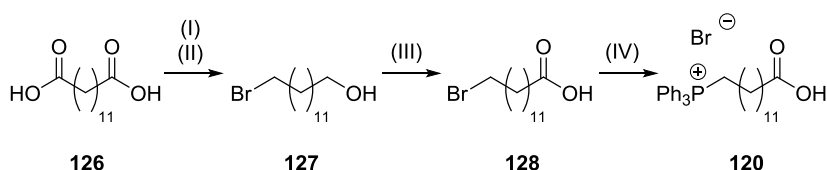
Schema 36: Retrosynthetische Überlegung zu Ancorinosid D (**76**).

Für die Synthese von **76** bzw. **116**, des Precursors der Lacey-Dieckmann-Zyklisierung, war somit das Meldrumsäureaddukt **118** (siehe Schema 36) der zentrale Baustein des Projekts. Analog zur Synthese von **75** wurde der Glykosylierungsdonor **100** (siehe Schema 32) nun an den kürzeren C11 Alkohol **122** (in Schema 36 rot dargestellt) unter Schmidt Bedingungen glykosyliert. Eine reduktive Debenzylierung des gebildeten Glykosids **123** mit TEMPO vermittelter Oxidation ergab die Galacturonsäure **124**. Wegen der bereits erwähnten Doppelbindung in Ancorinosid D (**76**), wurde anstelle eines Benzylester der Methylester **125** dargestellt. Ebenfalls analog zur Synthese von **75** konnte der Aldehyd **119** nach Desilylierung und Oxidation als erster Baustein synthetisiert werden (siehe Schema 37).


 Schema 37: Darstellung des Aldehyds **119**.

Reagenzien und Bedingungen: (I) TBSCl, Imidazol, CH₂Cl₂, rt, 16 h, 54 %; (II) MS 4 Å, TMSOTf, -40 °C → 0 °C, 2,5 h, 72%; (III) Pd/C, H₂, EtOAc, rt, 2,5 h, quant.; (IV) BAIB, TEMPO, CH₂Cl₂, H₂O, rt, 1 h, 86%; (V) K₂CO₃, MeI, DMF, 0 °C → rt, 2,5 h, 96%; (VI) AcOH, THF, H₂O, rt, 2 h, quant.; (VII) DMP, CH₂Cl₂, rt, 1 h, quant..

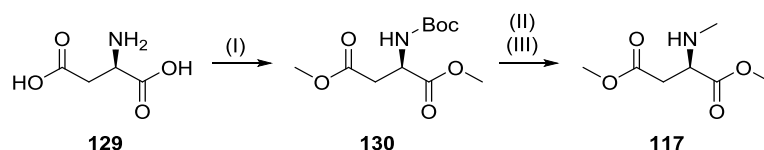
Die zweite, verbleibende C13 Hälfte der Seitenkette (in Schema 36 lila dargestellt) wurde ausgehend von Brassylsäure (**126**) synthetisiert. Diese wurde, nach Reduktion zum Diol und Monobromierung zum Alkohol **127**, zur Säure **128** oxidiert. Eine nucleophile Substitution mit PPh₃ lieferte das für die Darstellung der *cis*-Doppelbindung via Wittig Reaktion nötige Triphenylphosphonium Bromid **120** (siehe Schema 38). Die Verwendung dieser freien Carbonsäure **120** in einer Wittig Reaktion wurde gewählt, da der Einbau des C13 Fragments als Silyl-geschütztes Triphenylphosphoniumsalz in Vorversuchen sowohl eine längere Reaktionssequenz als auch nicht zufriedenstellende Gesamtausbeuten ergab.


 Schema 38: Darstellung des Phosphoniumsalzes **120**.

Reagenzien und Bedingungen: (I) LiAlH₄, THF, 0 °C → rt, 16 h, 88%; (II) HBr, Cyclohexan, reflux, 3 h, 67%; (III) BAIB, TEMPO, CH₂Cl₂, H₂O, rt, 16 h, 88%; (IV) PPh₃, MeCN, reflux, 4 d, quant..

Der für die geplante Aminolyse nötige letzte Baustein, das *N*-methylierte Dimethyl-D-aspartat (**117**) wurde, anders als für die Synthese von **75**, direkt aus D-Asparaginsäure **129** generiert. Hierzu wurde die ungeschützte D-Asparaginsäure (**129**) in einer Eintopfreaktion Reaktion zuerst Methyl verestert und Boc geschützt. Anschließend wurde zur Darstellung des gewünschten sekundären Amins **117** der

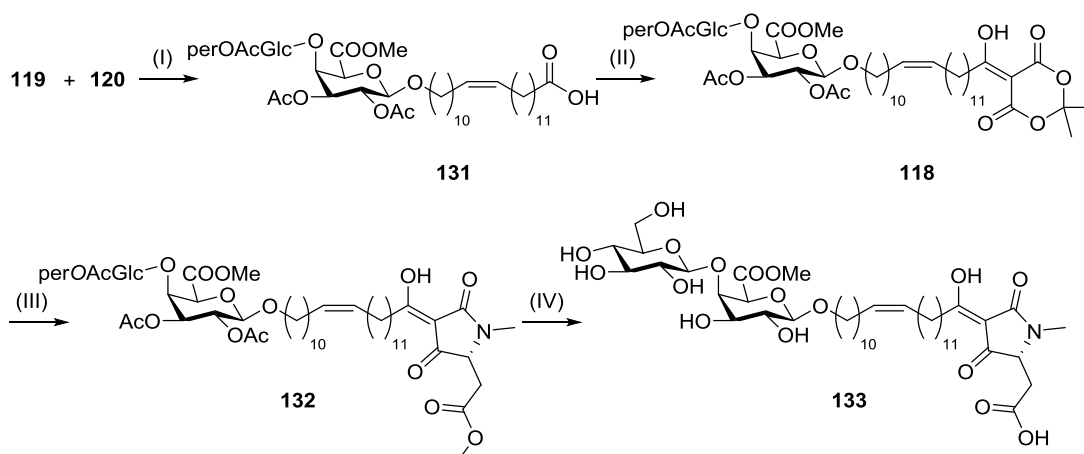
Stickstoff in **130** methyliert und analog zur Verbindung **98** das Carbamat abschließend sauer entfernt.



Schema 39: Darstellung des Aminosäurebausteins **117**.

Reagenzien und Bedingungen: (I) TMSCl, MeOH, 0 °C→rt, 16 h; (b) NEt₃, Boc₂O, 0 °C→rt, 16 h, 69 %; (II) MeI, NaH, DMF, 0 °C→rt, 1 h, 92%, (III) TFA, CH₂Cl₂, 0 °C rt, 1 h, 68%.

Das Zusammenfügen aller Teilbausteine zur Darstellung des natürlichen **76** begann mit der Kupplung von **119** und **120** in einer *cis*-selektiven Wittig Reaktion zur Generierung der vollständigen Seitenkette. Die daraus erhaltene Carbonsäure **131** wurde zur Acylierung von Meldrumsäure (**31**), als formalem C2 Fragment, unter Bildung des β -Ketoesters **118** verwendet. Dessen Aminolyse mit dem *N*-Methyl-D-Asparaginsäuredimethylester (**117**) führte, entgegen der Planung, nicht zum erwarteten Precursor, dem β -Ketoamid **116**, sondern direkt zur zyklisierten 3-Acyltetramsäure **132**.^[118,119] Eine solche Domino *N*-Acylierung-Zyklisierung wurde bisher in der Literatur noch nicht beobachtet.



Schema 40: Darstellung von Methyl-Ancorinosid D (**133**).

Reagenzien und Bedingungen: (I) NaHMDS, THF, -78 °C→rt, 2 h, 58%; (II) DMAP, EDCI, **31**, CH₂Cl₂, 0 °C→rt, 16 h, 68%; (III) **117**, PhMe, reflux, 4 h, 68%; (IV) NaOH, MeOH, CH₂Cl₂, rt, 30 min, 98 %.

Schließlich sollten alle Ester in der Tetramsäure **132** global verseift werden. Hierzu wurde zuerst das beispielsweise ebenfalls in der Totalsynthese von β -Lipomycin (**15**) erfolgreich verwendete wässrig methanolische LiOH verwendet. Dies führte aber zu einer Zersetzung des Substrates.^[42] Auch der Versuch einer mildereren

enzymatischen Esterspaltung sowohl mit Schweineleberesterase als auch mit Lipase Type II führte, vermutlich auf Grund der hohen Lipophilie des Substrats, auch nach bis zu zwei Wochen Reaktionszeit zu keinem erkennbaren Umsatz. Eine mildere Variante der basischen Verseifung mit methanolischen NaOH in CH_2Cl_2 führte zur Bildung des Methyluronats **133**. In Versuchen der Freisetzung des natürlichen **76** aus diesem Methyl-Ancorinosid D (**133**) kam es aber erneut zur Zersetzung des Substrates. Es liegt die Vermutung nahe, dass auf Grund der sterischen Umgebung des Galacturonsäureesters die Verseifung von **133** erschwert wird und nur langsam abläuft. Eine simple Substitution dieses Methylesters mit beispielsweise einem Trimethylsilylethylester sollte die Darstellung von **76** aber letztendlich möglich machen.

Dennoch konnte mit der Darstellung des Methyluronats **133** die Flexibilität der entwickelten Route zur Synthese der O-diglykosidischen 3-Acyltetramsäuren gezeigt werden. Die entwickelte Syntheseroute toleriert in den gezeigten Projekten sowohl eine Vielzahl unterschiedlicher Reaktionsbedingungen als auch Funktionalitäten. Außerdem kann sie wie an den beiden Beispielen gezeigt, leicht abgeändert werden, um etwaige Problemstellen zu umgehen. Die Route sollte somit auch auf die beiden verbliebenen Derivate Ancorinosid B und C anwendbar sein.

4 Literaturverzeichnis

- [1] D. A. Dias, S. Urban, U. Roessner, *Metabolites* **2012**, 2, 303–336.
- [2] S. M. Colegate, R. J. Molyneux, Eds., *Bioactive Natural Products: Detection, Isolation, and Structural Determination*, CRC Press, Boca Raton, **2008**.
- [3] P. M. Dewick, *Medicinal Natural Products: A Biosynthetic Approach*, Wiley, Chichester, West Sussex, England; New York, NY, USA, **2002**.
- [4] G. M. Cragg, D. J. Newman, *Pure Appl. Chem.* **2005**, 77, 7–24.
- [5] J. W.-H. Li, J. C. Vederas, *Science* **2009**, 325, 161–165.
- [6] C. Krishnamurti, S. C. Rao, *Indian J. Anaesth.* **2016**, 60, 861–862.
- [7] A. M. Gilson, M. A. Maurer, K. M. Ryan, M. Skemp-Brown, A. Husain, J. F. Cleary, *J. Pain Palliat. Care Pharmacother.* **2011**, 25, 246–251.
- [8] R. A. Maplestone, M. J. Stone, D. H. Williams, *Gene* **1992**, 115, 151–157.
- [9] M. S. Butler, *J. Nat. Prod.* **2004**, 67, 2141–2153.
- [10] D. T. Witiak, *J. Med. Chem.* **1996**, 39, 5289–5289.
- [11] M. S. Butler, *Nat. Prod. Rep.* **2008**, 25, 475–516.
- [12] A. Harvey, *Drug Discov. Today* **2008**, 13, 894–901.
- [13] J. N. Denis, A. E. Greene, D. Guenard, F. Gueritte-Voegelein, L. Mangatal, P. Potier, *J. Am. Chem. Soc.* **1988**, 110, 5917–5919.
- [14] K. M. Witherup, S. A. Look, M. W. Stasko, T. J. Ghiorzi, G. M. Muschik, G. M. Cragg, *J. Nat. Prod.* **1990**, 53, 1249–1255.
- [15] J. Hong, *Chem. - Eur. J.* **2014**, 20, 10204–10212.
- [16] E. J. Corey, W. J. Howe, H. W. Orf, D. A. Pensak, G. Petersson, *J. Am. Chem. Soc.* **1975**, 97, 6116–6124.
- [17] R. Schobert, A. Schlenk, *Bioorg. Med. Chem.* **2008**, 16, 4203–4221.
- [18] G. Athanasellis, O. Igglessi-Markopoulou, J. Markopoulos, *Bioinorg. Chem. Appl.* **2010**, 2010, 1–11.
- [19] B. J. L. Royles, *Chem. Rev.* **1995**, 95, 1981–2001.
- [20] P. S. Steyn, P. L. Wessels, *Tetrahedron Lett.* **1978**, 47, 4707–4710.
- [21] M. J. Nolte, P. S. Steyn, P. L. Wessels, *J. Chem. Soc. Perkin Trans. 1* **1980**, 1051–1056.
- [22] T. Yamaguchi, K. Saito, T. Tsujimoto, H. Yuki, *J. Heterocycl. Chem.* **1976**, 13, 533–537.

- [23] M.-H. Lebrun, P. Duvert, F. Gaudemer, A. Gaudemer, C. Deballon, P. Boucly, *J. Inorg. Biochem.* **1985**, 24, 167–181.
- [24] R. T. Gallagher, J. L. Richard, H. M. Stahr, R. J. Cole, *Mycopathologia* **1978**, 66, 31–36.
- [25] H. Kohl, S. V. Bhat, J. R. Patell, N. M. Gandhi, J. Nazareth, P. V. Divakar, N. J. Souza, *Tetrahedron Lett.* **1974**, 12, 983–986.
- [26] N. D. Davis, U. L. Diener, G. Morgan-Jones, *Appl. Environ. Microbiol.* **1977**, 34, 155–157.
- [27] R. J. Capon, C. Skene, E. Lacey, J. H. Gill, D. Wadsworth, T. Friedel, *J. Nat. Prod.* **1999**, 62, 1256–1259.
- [28] M. Kawada, Y. Yoshimoto, H. Kumagai, T. Someno, I. Momose, N. Kawamura, K. Isshiki, D. Ikeda, *J. Antibiot.* **2004**, 57, 235–237.
- [29] W. O. Foye, *J. Pharm. Sci.* **1961**, 50, 93–108.
- [30] S. Donadio, M. Staver, J. McAlpine, S. Swanson, L. Katz, *Science* **1991**, 252, 675–679.
- [31] J. Staunton, K. J. Weissman, *Nat. Prod. Rep.* **2001**, 18, 380–416.
- [32] M. A. Marahiel, T. Stachelhaus, H. D. Mootz, *Chem. Rev.* **1997**, 97, 2651–2674.
- [33] D. Konz, M. A. Marahiel, *Chem. Biol.* **1999**, 6, R39–R48.
- [34] D. Schwarzer, R. Finking, M. A. Marahiel, *Nat. Prod. Rep.* **2003**, 20, 275–287.
- [35] F. Hemmerling, F. Hahn, *Beilstein J. Org. Chem.* **2016**, 12, 1512–1550.
- [36] D. Boettger, C. Hertweck, *ChemBioChem.* **2013**, 14, 28–42.
- [37] C. Gui, Q. Li, X. Mo, X. Qin, J. Ma, J. Ju, *Org. Lett.* **2015**, 17, 628–631.
- [38] T. B. Kakule, D. Sardar, Z. Lin, E. W. Schmidt, *ACS Chem. Biol.* **2013**, 8, 1549–1557.
- [39] C. Bihlmaier, E. Welle, C. Hofmann, K. Welzel, A. Vente, E. Breitling, M. Muller, S. Glaser, A. Bechthold, *Antimicrob. Agents Chemother.* **2006**, 50, 2113–2121.
- [40] C. J. Thibodeaux, C. E. Melançon, H. Liu, *Angew. Chem. Int. Ed.* **2008**, 47, 9814–9859.
- [41] D. H. Kwan, P. F. Leadlay, *ACS Chem. Biol.* **2010**, 5, 829–838.
- [42] O. Hartmann, M. Kalesse, *Angew. Chem. Int. Ed.* **2014**, 53, 7335–7338.

- [43] P. Caffrey, *ChemBioChem*. **2003**, 4, 654–657.
- [44] R. Reid, M. Piagentini, E. Rodriguez, G. Ashley, N. Viswanathan, J. Carney, D. V. Santi, C. R. Hutchinson, R. McDaniel, *Biochemistry* **2003**, 42, 72–79.
- [45] S. Sakuda, M. Ono, H. Ikeda, Y. Inagaki, J. Nakayama, A. Suzuki, A. Isogai, *Tetrahedron Lett.* **1997**, 38, 7399–7402.
- [46] G. F. Crum, W. H. Devries, T. E. Eble, C. M. Large, J. W. Shell, *Antibiot. Annu.* **1955**, 3, 893–896.
- [47] G. Cassani, R. R. Burgess, H. M. Goodman, L. Gold, *Nature. New Biol.* **1971**, 230, 197–200.
- [48] S. Zorov, Y. Yuzenkova, V. Nikiforov, K. Severinov, N. Zenkin, *Antimicrob. Agents Chemother.* **2014**, 58, 1420–1424.
- [49] C. Siddhikol, J. W. Erbstoeszer, B. Weisblum, *J. Bacteriol.* **1969**, 99, 151–155.
- [50] M. Isaka, P. Chinthanom, S. Supothina, P. Tobwor, N. L. Hywel-Jones, *J. Nat. Prod.* **2010**, 73, 2057–2060.
- [51] S. Bruckner, U. Bilitewski, R. Schobert, *Org. Lett.* **2016**, 18, 1136–1139.
- [52] B. H. Howard, H. Raistrick, *Biochem. J.* **1954**, 57, 212–222.
- [53] B. H. Howard, H. Raistrick, *Biochem. J.* **1949**, 44, 227–233.
- [54] J. Shoji, S. Shibata, U. Sankawa, H. Taguchi, Y. Shibanuma, *Chem. Pharm. Bull.* **1965**, 13, 1240–1246.
- [55] J. A. Beutler, B. D. Hilton, P. Clark, M. S. Tempesta, D. G. Corley, *J. Nat. Prod.* **1988**, 51, 562–566.
- [56] I. Casser, B. Steffan, W. Steglich, *Angew. Chem. Int. Ed.* **1987**, 26, 586–587.
- [57] S. V. Ley, S. C. Smith, P. R. Woodward, *Tetrahedron Lett.* **1992**, 48, 1145–1174.
- [58] R. F. Vesonder, L. W. Tjarks, W. K. Rohwedder, H. R. Burmeister, J. A. Laugal, *J. Antibiot.* **1979**, 32, 759–761.
- [59] S. B. Singh, D. L. Zink, M. A. Goetz, A. W. Dombrowski, J. D. Polishook, D. J. Hazuda, *Tetrahedron Lett.* **1998**, 39, 2243–2246.
- [60] J. W. Sims, J. P. Fillmore, D. D. Warner, E. W. Schmidt, *Chem. Commun.* **2005**, 186–188.
- [61] K. Jomon, Y. Kuroda, M. Ajisaka, H. Sakai, *J. Antibiot.* **1972**, 25, 271–280.

- [62] J. Stöckl, Modellstudien Zur Entwicklung einer neuen Synthesemethode für polycyclische Tetramsäure-Makrolactame, Dissertation, Universität Bayreuth, **2017**.
- [63] J. A. V. Blodgett, D.-C. Oh, S. Cao, C. R. Currie, R. Kolter, J. Clardy, *Proc. Natl. Acad. Sci.* **2010**, *107*, 11692–11697.
- [64] S. Saha, W. Zhang, G. Zhang, Y. Zhu, Y. Chen, W. Liu, C. Yuan, Q. Zhang, H. Zhang, L. Zhang, *et al.*, *Chem. Sci.* **2017**, *8*, 1607–1612.
- [65] Y. Luo, H. Huang, J. Liang, M. Wang, L. Lu, Z. Shao, R. E. Cobb, H. Zhao, *Nat. Commun.* **2013**, *4*, 480–485.
- [66] M. Bertasso, M. Holzenkämpfer, A. Zeeck, E. Stackebrandt, W. Beil, H.-P. Fiedler, *J. Antibiot.* **2003**, *56*, 364–371.
- [67] P. R. Graupner, A. Carr, E. Clancy, J. Gilbert, K. L. Bailey, J.-A. Derby, B. C. Gerwick, *J. Nat. Prod.* **2003**, *66*, 1558–1561.
- [68] P. Jouin, B. Castro, D. Nisato, *J. Chem. Soc., Perkin Trans. 1* **1987**, 1177–1182.
- [69] A. S. Ivanov, *Chem. Soc. Rev.* **2008**, *37*, 789–811.
- [70] K. Janikowska, J. Rachoń, S. Makowiec, *Russ. Chem. Rev.* **2014**, *83*, 620–637.
- [71] J. Jiang, W.-R. Li, R. M. Przeslawski, M. M. Joullie, *Tetrahedron Lett.* **1993**, *34*, 6705–6708.
- [72] D. Ma, J. Ma, W. Ding, L. Dai, *Tetrahedron: Asymmetry* **1996**, *7*, 2365–2370.
- [73] M. Hosseini, H. Kringelum, A. Murray, J. E. Tønder, *Org. Lett.* **2006**, *8*, 2103–2106.
- [74] J. Löffler, R. Schobert, *J. Chem. Soc., Perkin Trans. 1* **1996**, 2799–2802.
- [75] H. J. Bestmann, D. Sandmeter, *Angew. Chem.* **1975**, *87*, 630–630.
- [76] R. Schobert, *Naturwissenschaften* **2006**, *94*, 1–11.
- [77] R. Schobert, C. Jagusch, C. Melanophy, G. Mullen, *Org. Biomol. Chem.* **2004**, *2*, 3524–3529.
- [78] R. Schobert, M. Dietrich, G. Mullen, J.-M. Urbina-Gonzalez, *Synthesis* **2006**, 3902–3914.
- [79] R. Schobert, C. Jagusch, *Tetrahedron* **2005**, *61*, 2301–2307.
- [80] G. Martinez-Ariza, M. Ayaz, S. A. Roberts, W. A. Rabanal-León, R. Arratia-Pérez, C. Hulme, *Angew. Chem. Int. Ed.* **2015**, *54*, 11672–11676.

- [81] B. K. Singh, S. S. Bisht, R. P. Tripathi, *Beilstein J. Org. Chem.* **2006**, 24, 1–6.
- [82] B. Gabriele, P. Plastina, G. Salerno, M. Costa, *Synlett.* **2005**, 935–938.
- [83] T. Ishida, R. Kobayashi, T. Yamada, *Org. Lett.* **2014**, 16, 2430–2433.
- [84] R. C. F. Jones, G. E. Peterson, *Tetrahedron Lett.* **1983**, 24, 4751–4754.
- [85] J. L. Bloomer, F. E. Kappler, *J. Chem. Soc., Perkin Trans. 1* **1976**, 1485–1491.
- [86] R. C. F. Jones, S. Sumaria, *Tetrahedron Lett.* **1978**, 34, 3173–3176.
- [87] R. C. F. Jones, G. E. Peterson, *Tetrahedron Lett.* **1983**, 24, 4757–4760.
- [88] R. C. F. Jones, M. J. Begley, G. E. Peterson, S. Sumaria, *J. Chem. Soc., Perkin Trans. 1* **1990**, 1959–1968.
- [89] K. Hori, M. Arai, K. Nomura, E. Yoshii, *Chem. Pharm. Bull.* **1987**, 35, 4368–4371.
- [90] J. L. Van Der Baan, J. W. F. K. Barnick, F. Bickelhaupt, *Tetrahedron* **1978**, 34, 223–231.
- [91] K. Fries, G. Finck, *Berichte Dtsch. Chem. Ges.* **1908**, 41, 4271–4284.
- [92] Y.-C. Jeong, M. G. Moloney, *J. Org. Chem.* **2011**, 76, 1342–1354.
- [93] T. Sengoku, J. Wierzejska, M. Takahashi, H. Yoda, *Synlett.* **2010**, 2944–2946.
- [94] T. Sengoku, Y. Nagae, Y. Ujihara, M. Takahashi, H. Yoda, *J. Org. Chem.* **2012**, 77, 4391–4401.
- [95] A. Schlenk, R. Diestel, F. Sasse, R. Schobert, *Chem. - Eur. J.* **2010**, 16, 2599–2604.
- [96] R. N. Lacey, *J. Chem. Soc.* **1954**, 850–854.
- [97] J. P. Schaefer, J. J. Bloomfield, in *Org. React.* (Ed.: John Wiley & Sons, Inc.), John Wiley & Sons, Inc., Hoboken, NJ, USA, **2011**, pp. 1–203.
- [98] S. V. Ley, P. R. Woodward, *Tetrahedron Lett.* **1987**, 28, 3019–3020.
- [99] S. V. Ley, S. C. Smith, P. R. Woodward, *Tetrahedron Lett.* **1988**, 29, 5829–5832.
- [100] C. S. Pak, H. C. Yang, E. B. Choi, *Synthesis* **1992**, 1213–1214.
- [101] S. P. Raillard, G. Ji, A. D. Mann, T. A. Baer, *Org. Process Res. Dev.* **1999**, 3, 177–183.

- [102] J. Poncet, P. Jouin, B. Castro, L. Nicolas, M. Boutar, A. Gaudemer, *J. Chem. Soc., Perkin Trans. 1* **1990**, 611-616.
- [103] R. Böhme, G. Jung, E. Breitmaier, *Helv. Chim. Acta* **2005**, 88, 2837–2841.
- [104] T. Yoshinari, K. Ohmori, M. G. Schrems, A. Pfaltz, K. Suzuki, *Angew. Chem. Int. Ed.* **2010**, 49, 881–885.
- [105] L. T. Burke, D. J. Dixon, S. V. Ley, F. Rodriguez, *Org. Biomol. Chem.* **2005**, 3, 274–280.
- [106] D. A. Longbottom, A. J. Morrison, D. J. Dixon, S. V. Ley, *Angew. Chem. Int. Ed.* **2002**, 41, 2786–2790.
- [107] X. Wang, Z. Guo, *Chem. Rev. Soc.* **2013**, 42, 202–224.
- [108] J. A. Jaurigue, P. H. Seeberger, *Front. Cell. Infect. Microbiol.* **2017**, 7, 1–12.
- [109] A. David, *Isr. J. Chem.* **2010**, 50, 204–219.
- [110] M. U. Ahmad, S. M. Ali, A. Ahmad, S. Sheikh, P. Chen, I. Ahmad, *Lipid Technol.* **2015**, 27, 223–226.
- [111] U. Gerhard, J. P. Mackay, R. A. Maplestone, D. H. Williams, *J. Am. Chem. Soc.* **1993**, 115, 232–237.
- [112] J. Beiger, Total Synthesis of Aflastatin A, Dissertation, Harvard University, **2013**.
- [113] S. Loscher, R. Schobert, *Chem. Eur. J.* **2013**, 19, 10619–10624.
- [114] M. L. Hofferberth, R. Brückner, *Angew. Chem.* **2014**, 126, 7456–7462.
- [115] S. V. Pronin, S. A. Kozmin, *J. Am. Chem. Soc.* **2010**, 132, 14394–14396.
- [116] C. Schmidt, *Annalen der Chemie und Pharmacie*, Winter, Heidelberg, **1844**.
- [117] H. Rabinowitz, S. Vogel, Eds., *The Manual of Scientific Style: A Guide for Authors, Editors, and Researchers*, Elsevier/Academic Press, Amsterdam; Burlington, MA, **2009**.
- [118] S. Bruckner, R. G. Haase, R. Schobert, *Chem. - Eur. J.* **2017**, 23, 5692–5695.
- [119] K. Lovmo, S. Dütz, M. Harras, R. G. Haase, W. Milius, R. Schobert, *Tetrahedron Lett.* **2017**, 58, 4796–4798.

5 Darstellung des Eigenanteils

Die in der vorliegenden Dissertation beschriebenen und abgedruckten Publikationen sind in Zusammenarbeit mit anderen Wissenschaftlern erarbeitet und ausgearbeitet worden. Der Eigenanteil an den jeweiligen Veröffentlichungen soll im Folgenden detailliert dargestellt werden.

5.1 Publikation zur Totalsynthese von Aurantosid G

Diese Arbeit wurde im Journal *Angewandte Chemie Internationale Edition* (*Angew. Chem. Int. Ed.* **2016**, 55, 10122-10125/ DOI:10.1002/anie.201604912) veröffentlicht und liegt vor unter dem Titel:

**„Total Synthesis of Aurantoside G, an *N*- β -Glycosylated
3-Oligoenoyltetramic Acid from *Theonella swinhoei*“**

(siehe Kapitel 3.1.1 bzw. 6.1)

Von den Autoren *Markus Petermichl, Sebastian Loscher und Rainer Schobert*.

Die Synthesestrategie wurde von Dr. Sebastian Loscher und mir in Zusammenarbeit erarbeitet. Aufgetretene Probleme während der Synthese wurden in gemeinschaftlicher Zusammenarbeit zwischen Prof. Dr. Rainer Schobert, Dr. Sebastian Loscher und mir in wissenschaftlichen Diskussionen erörtert und gelöst. Synthetische Arbeiten, die anfallende Analytik der Präparate sowie deren Auswertung wurden von mir durchgeführt.

Wissenschaftlichen Diskussionen zu dem Thema fanden zwischen Prof. Dr. Rainer Schobert, Dr. Sebastian Loscher und mir statt.

Prof. Dr. Rainer Schobert und ich waren an der Verfassung, der Diskussion sowie der Korrektur und Revision des Manuskripts beteiligt.

5.2 Publikation zur Totalsynthese von Ancorinosid A

Diese Arbeit wurde im Journal *Chemistry A European Journal* (*Chem. Eur. J.* **2017**, 23, 14743-14746/DOI: 10.1002/chem.201704379) veröffentlicht und liegt vor unter dem Titel:

„Total Synthesis of Diglycosidic Tetramic Acid Ancorinoside A“

(siehe Kapitel 3.2.1 bzw. 6.2)

Von den Autoren *Markus Petermichl und Rainer Schobert*.

Die Synthesestrategie wurde von mir erarbeitet. Aufgetretene Probleme während der Synthese wurden in wissenschaftlichen Diskussionen zwischen Prof. Dr. Rainer Schobert und mir erörtert und gelöst. Synthetische Arbeiten, die anfallende Analytik der Präparate sowie deren Auswertung wurden von mir durchgeführt.

Prof. Dr. Rainer Schobert und ich waren an der Verfassung, der Diskussion sowie der Korrektur und Revision des Manuskripts beteiligt.

5.3 Publikation zur Synthese von Methyl-Ancorinosid D

Diese Arbeit wurde im Journal *Synthesis* (*Synthesis*, **2019**, 51, 730-738/DOI: 10.1055/s-0037-1610287) veröffentlicht und liegt vor unter dem Titel:

„A Synthetic Route to the MT1-MMP Inhibitor Ancorinoside D“

(siehe Kapitel 3.2.2 bzw. 6.3)

Von den Autoren *Markus Petermichl, Christine Steinert und Rainer Schobert*.

Die Syntheseplanung wurde von mir erarbeitet. Synthetische Arbeiten, die Analytik der Präparate sowie deren Auswertung wurden von Christine Steinert und mir durchgeführt. Aufgetretene Probleme während der Synthese wurden in wissenschaftlichen Diskussionen zwischen Prof. Dr. Rainer Schobert, Christine Steinert und mir erörtert und gelöst. Die Synthesen der Monosaccharide sowie die Synthese der Tetransäure und Entschützungen wurden von mir durchgeführt. glykosidische Bindungsknüpfungen und Versuche zu Olefinierungsreaktionen wurden von Christine Steinert durchgeführt.

Prof. Dr. Rainer Schobert, Christine Steinert und ich waren an der Verfassung, der Diskussion sowie der Korrektur und der Revision des Manuskripts beteiligt.

6 Publikationen

6.1 Publikation I

Total Synthesis of Aurantoside G, an *N*- β -Glycosylated 3-Oligoenoyltetramic Acid from *Theonella swinhoei*

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Natural Products

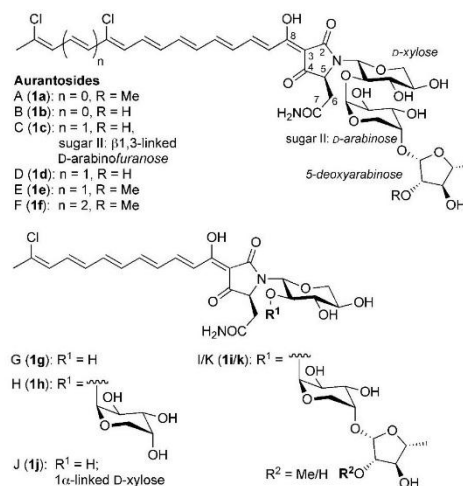
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German Edition: DOI: 10.1002/ange.201604912Total Synthesis of Aurantoside G, an N-β-Glycosylated 3-Oligoenoyltetramic Acid from *Theonella swinhoei*

Markus Petermichl, Sebastian Loscher, and Rainer Schobert*

Abstract: The first synthesis of a natural N-glycosylated 3-acyltetramic acid is reported. Aurantoside G (**1g**), a deep-red metabolite of the marine sponge *Theonella swinhoei*, is highly delicate in the pure state. It features a chlorinated dodecapentaenoyl side chain at an L-asparagine-derived tetramic acid, the ring nitrogen atom of which is linked to a β-configured D-xylose. The side chain was built through consecutive Wittig and HWE reactions and used to N-acylate the amino group of an asparagine that had already been N-xylosylated through a Fukuyama–Mitsunobu reaction. This N-acylation step fixes the β-configuration of the xylose, which is essential for the antifungal activity, but only if the sugar carries bulky, electron-rich protecting groups such as PMB. In the final step, the heterocycle was closed quantitatively through a basic Lacey–Dieckmann condensation of an entirely unprotected precursor.

Natural 3-acyltetramic acids (3-acylpyrrolidine-2,4-diones) are hybrid polyketide/amino acid metabolites that are produced by bacteria, molds, fungi, and sponges, and which show a high incidence and broad spectrum of biological activities.^[1] Owing to the flexibility of the polyketide biosynthesis machinery, they come in many structural variants and complexities. Some of them include sugar residues. While the majority of these compounds are O-glycosylated at the end of the 3-acyl side-chain, such as the epicoccamides^[2] and ancorinosides,^[3] others feature C-glycosylation at this position, like the aflastatins,^[4] or N-glycosylation at the lactam nitrogen atom, as in the aurantosides^[5] and rubrosides.^[6] Only a few total syntheses of glycotetramates, and none of N-glycosylated tetramic acids, have been reported, so far.^[7] All known aurantosides A–K (**1a–k**) have been isolated as orange-red pigments from lithistid marine sponges of the genera *Theonella* (**1a**,^[5a,b] **1b**,^[5a] **1g–i**,^[5d] **1j**,^[5c] *Homophymia* (**1c**),^[5c] *Siliquariaspongia* (**1d–f**),^[5f] or *Melophlus* (**1k**).^[5g] They share an asparagine-derived tetramic acid that carries a mono- or dichlorinated polyenoyl residue at C-3, which is N-glycosylated with either D-xylose or di- and trisaccharides comprised of D-xylose, D-arabinose, and 5-deoxyarabinofuranose. Some aurantosides show a distinct antifungal effect that is not clearly related to their structures. Aurantosides A, B, E and I are efficacious against *Candida albicans* (wildtype) at minimal inhibitory concentrations (MIC) of 1 μg mL⁻¹ or below, while aurantosides D, G and K show MIC values of

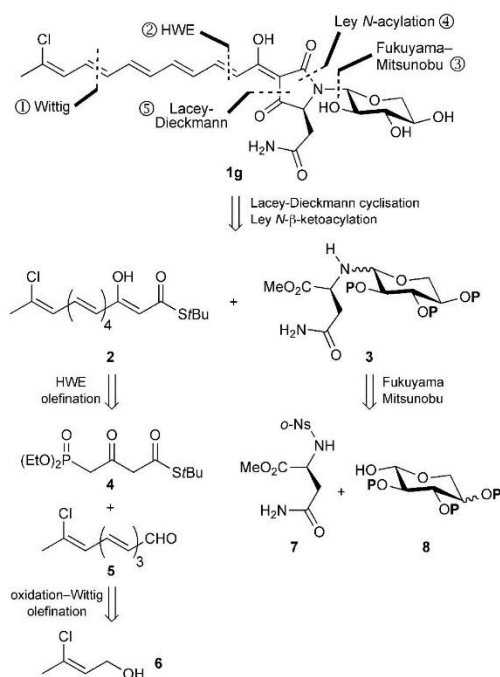
between 2 and 10 μg mL⁻¹. The aurantosides F, H, and J are virtually inactive and aurantoside C has not been tested yet. The mode of antifungal action might be related to that of the polyene macrolides of the nystatin type. The few tests for antimicrobial activity have mostly been disappointing.



Any synthetic approach to the aurantosides has to address the issues of their inherent instability,^[5c] and the necessity to control the β-configuration of the N-glycosidic linkage. The latter seems to play a role in the antifungal effect, which is apparent from the activity difference between the epimers **1g** and **1j**. We now developed a route to aurantoside G (**1g**), the key steps of which should also be applicable to the syntheses of its more complex congeners. Our retrosynthetic approach is outlined in Scheme 1. To avoid problems associated with the polarity and metal-chelating propensity of 3-acyltetramic acids, this functionality was to be generated only in a final base-induced Lacey–Dieckmann^[8] cyclization of the fully functionalized N-(D-xylosyl)-N-(β-ketotetradecapentaenoyl)asparagine. The latter should be prepared by aminolysis of thioester **2**, as the source of the entire 3-acyl side-chain, with methyl N-D-xylosylasparagine (**3**) according to a method reported by Ley et al.^[9] We intended to build thioester **2** through HWE olefination of the known β-ketophosphonate **4** with the chlorinated tetraenal **5**, which should be accessible through a Wittig olefination of carboxy-thoxymethylenetriphenylphosphorane with the aldehyde 3-chlorobut-(2Z)-enal, ideally prepared in situ through oxida-

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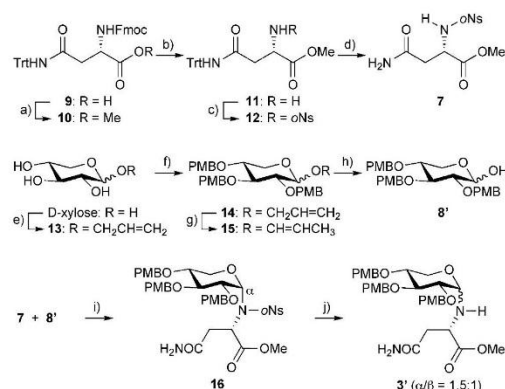


Scheme 1. Retrosynthetic approach to auranoside G (**1g**). *o*Ns = *o*-(O_2N) $\text{C}_6\text{H}_4\text{SO}_2$.

tion of alcohol **6**. The *N*-xylosylasparaginate **3** was to be prepared by a Fukuyama–Mitsunobu reaction of a tris-protected *D*-xylose **8** with *N*-nosylated methyl asparaginate **7**. It was unclear at this point whether the bulky *o*-nosyl group would give rise to α - or β -glycosylation and whether such a preferred configuration might be preserved throughout the synthesis.

We started out with the synthesis of the unknown methyl *N*-(*o*-nosyl)asparaginate **7** from commercially available bis-protected asparagine **9** in four steps and 80% overall yield.^[10] The known intermediates, that is, the bisprotected methyl ester **10** and the α -aminoester **11**, were obtained for the first time in a pure crystalline form rather than as oils^[11] (Scheme 2, top row).

It took some experimentation to identify *p*-methoxybenzyl (PMB) as the optimum protecting group for xylose. PMB as an electron-releasing group enables the Fukuyama–Mitsunobu reaction of *D*-xylose with the electron-poor, weakly nucleophilic amine **7**. Moreover, PMB is bulky enough to direct the subsequent *N*-acylation of **3** with **2** in favor of the β -configuration. The new PMB-protected xylose **8'** was prepared from *D*-xylose in four steps and 58% yield as a 1.7:1.0 mixture of α - and β -anomers. Selective 1-*O*-allylation^[12] of *D*-xylose and subsequent benzylation of the resulting glycoside **13** with PMBCl furnished the fully protected xylose **14**. The allyl ether was cleaved^[13] by first isomerizing it with base to the corresponding enol ether **15**, which was hydrolyzed right

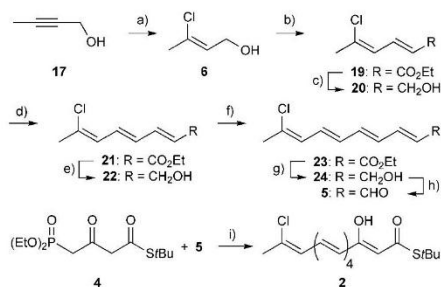


Scheme 2. Synthesis of **3'**. Reagents and conditions: a) K_2CO_3 , MeI, DMF, $0^\circ\text{C} \rightarrow \text{RT}$, 1 h; 99%. b) piperidine, DMF, RT, 1 h; 99%. c) *o*NsCl, NEt_3 , CH_2Cl_2 , $0^\circ\text{C} \rightarrow \text{RT}$, 16 h; 92%. d) $\text{F}_3\text{CCO}_2\text{H}$, CH_2Cl_2 , $0^\circ\text{C} \rightarrow \text{RT}$, 1 h; 89%. e) allylic alcohol, $\text{BF}_3 \cdot \text{OEt}_2$, reflux, 16 h; 77%. f) PMBCl, NaH, DMF, $0^\circ\text{C} \rightarrow \text{RT}$, 72 h; 86%. g) KOtBu , DMF, 70°C , 2 h. h) 0.1 M HCl, acetone, reflux, 1 h; 87% over 2 steps. i) DIAD, PPh_3 , THF, $-78^\circ\text{C} \rightarrow \text{RT}$, 16 h; 69%. j) DIPEA, PhSH, DMF, RT, 4 h; 77%. DMF = *N,N*-dimethylformamide, DIAD = diisopropyl azodicarboxylate, DIPEA = *N,N*-diisopropylamine, Fmoc = fluorenylmethoxycarbonyl, Trt = triphenylmethyl.

away with hydrochloric acid to leave building block **8'** (Scheme 2, middle row).

The Fukuyama–Mitsunobu reaction^[14] of **8'** with sulfonamide **7** proceeded fast and with complete consumption of the starting materials. The *N*-nosylated product amine **16** was isolated in 69% yield as the pure α -anomer, as apparent from the NMR spectra. The chemical shifts of the C-1 of the xylose differed by ca. $\Delta\delta = 20$ ppm for α - and β -anomers, and the coupling constants $^3J_{\text{HH}}$ for 1-H were 3–4 Hz for the α -anomer and 7–8 Hz for the β -anomer. De-nosylation of **16** with thiophenol and Hünig's base afforded the key amine **3'** ($\text{P} = \text{PMB}$) in 77% yield, albeit as a 1.5:1.0 mixture of α - and β -anomers (Scheme 2, bottom).

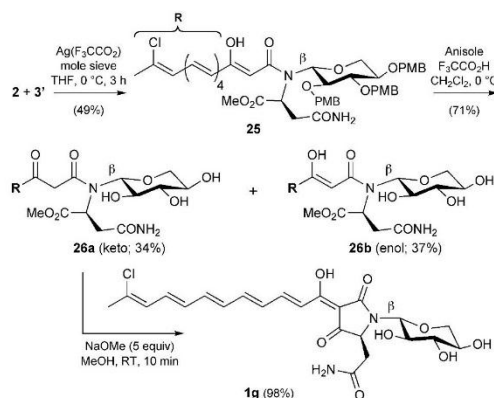
Next, thioester **2** was synthesized starting from 3-chlorobut-(2*Z*)-en-1-ol (**6**), which was prepared through the reaction of but-2-yn-1-ol (**17**) with Red-Al and NCS, according to an improved adaptation of the method of Poulter et al.^[15] (Scheme 3). We began the stepwise chain elongation of alcohol **6** by employing a domino oxidation/Wittig olefination, as described by Taylor et al.,^[16] using MnO_2 and carboethoxymethylenetriphenylphosphorane (**18**), since the corresponding aldehyde of **6** is rather delicate. The product ethyl dienoate **19** was reduced to the alcohol **20**, and the chain was lengthened in the same manner by domino oxidation/Wittig reaction with MnO_2 and ylide **18** to give the ethyl trienoate **21**. After a third sequence of reduction and domino oxidation/Wittig reaction, the ethyl tetraenoate **23** was obtained. It was reduced to the corresponding alcohol **24**, which was finally oxidized with MnO_2 to the desired aldehyde **5**. Because of its instability, even when kept under an inert gas atmosphere in a dark freezer at -20°C , this aldehyde was submitted right away to a HWE reaction with Ley's *S*-tert-



Scheme 3. Synthesis of **2**. Reagents and conditions: a) i) Red-Al, THF, 0 °C → RT, 16 h; ii) NCS, THF, −78 °C → 0 °C, 3 h; 82%. b) MnO₂, Ph₃P=CHCO₂Et (**18**), CH₂Cl₂, reflux, 16 h; 63%. c) DIBAL-H, CH₂Cl₂, −78 °C, 1 h; 75%. d) MnO₂, **18**, CH₂Cl₂, reflux, 16 h; 77%. e) DIBAL-H, CH₂Cl₂, −78 °C, 1 h; 94%. f) MnO₂, **18**, CH₂Cl₂, reflux, 16 h; 60%. g) DIBAL-H, CH₂Cl₂, −78 °C, 1 h; 77%. h) MnO₂, CH₂Cl₂, RT, 3 h. i) NaH, THF, 0 °C, 3 h; 84% over 2 steps. Red-Al = sodium bis(2-methoxyethoxy)aluminumhydride, THF = tetrahydrofuran, DIBAL-H = diisobutylaluminumhydride.

butyl 4-(diethylphosphono)-3-oxobutanethioate (**4**)^[17] to afford the thioester **2** in 84% yield (11% over nine steps).

The aminolysis of thioester **2** with a 1.5:1.0 mixture of α - and β -anomers of methyl *N*-D-xylosylasparaginate **3'** in the presence of an excess of silver trifluoroacetate according to Ley's general method^[7a,9] afforded the β -ketoamide **25** as a pure β -isomer in 49% yield with respect to recovered unreacted **3'** (Scheme 4). We assume that only the β -anomer



Scheme 4. Final steps to auranoside **G** (**1g**): β -selective Ley *N*-(β -keto)acylation to give **25**, followed by deprotection and Lacey–Dieckmann cyclization.

of **3'** enters into a reaction with **2** owing to steric shielding of the α -anomer by the PMB groups at the xylose, and that epimerization of the residual α -anomer is not fast enough under the reaction conditions. This epimerization definitely took place upon the recovery (extraction, concentration) of unreacted **3'**, which again yielded a mixture of both anomers,

ready to be reacted once more. Several reaction cycles are necessary to completely convert **3'** into β -ketoamide **25**. Since all auranosides except **1j** feature a β -xylose bearing a bulky arabinose at C-2, the above route to auranoside **G** (**1g**) should also be applicable to the β -selective synthesis of these congeners. Cleavage of the PMB groups of **25** with trifluoroacetic acid afforded the unprotected *N*-glycosylated β -ketoamide **26** as a separable 1:1 mixture of keto and enol tautomers. Only the keto tautomer **26a** underwent Lacey–Dieckmann cyclization which, when stopped after 10 min reaction time, furnished the chemically and enantiomerically pure auranoside **G** (**1g**) in virtually quantitative yield. The enol tautomer **26b** needed to be re-equilibrated with acid to give the initial 1:1 mixture of both epimers. Like other polyenoyltetramic acids such as β -lipomycin,^[5c,7f] pure synthetic auranoside **G** proved unstable and prone to decomposition, which thwarted further biological tests.

In summary, the sponge metabolite auranoside **G** (**1g**) was synthesized as the first example of an *N*-glycosylated 3-acyltetramic acid in 3.7% overall yield. The β -configuration of the D-xylose residue, which is essential for its bioactivity, was fixed only in the course of the penultimate Ley-type *N*-acylation step. The β -selectivity is governed by the bulky PMB substituents on the xylose. The electron-releasing effect of the PMB residues was also exploited to promote the preceding Fukuyama–Mitsunobu glycosylation step. We expect this synthetic approach to be applicable also to the other auranosides, which all bear bulky mono- or oligosaccharides at C-2 of a β -xylose. We also demonstrated that Lacey–Dieckmann cyclization may serve as the final step in the synthesis of even delicate tetramic acids, since it does not lead to racemization or interfere with extended conjugated polyene fragments or unprotected sugars.

Keywords: auranosides · glycoconjugates · natural products · tetramic acids · total synthesis

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- [1] Reviews on tetramic acids: a) H.-G. Henning, A. Gelbin, *Adv. Heterocycl. Chem.* **1993**, *57*, 139–185; b) B. J. L. Royle, *Chem. Rev.* **1995**, *95*, 1981–2001; c) E. L. Ghisalberti in *Studies in Natural Products Chemistry*, Vol. 28/1 (Ed.: Atta-ur-Rahman), Elsevier, Dordrecht, **2003**, pp. 109–163; d) “Monopyrrolic natural compounds including tetramic acid derivatives”: A. Gossauer in *Progress in the Chemistry of Organic Natural Products*, Vol. 86 (Eds.: W. Herz, H. Falk, G. W. Kirby), Springer, Wien, **2003**, pp. 1–188; e) R. Schobert, *Naturwissenschaften* **2007**, *94*, 1–11; f) R. Schobert, A. Schlenk, *Bioorg. Med. Chem.* **2008**, *16*, 4203–4221.
- [2] a) A. D. Wright, C. Osterhage, G. M. König, *Org. Biomol. Chem.* **2003**, *1*, 507–510; b) H. V. K. Wangun, H.-M. Dahse, C. Hertweck, *J. Nat. Prod.* **2007**, *70*, 1800–1803.
- [3] a) S. Ohta, E. Ohta, S. Ikegami, *J. Org. Chem.* **1997**, *62*, 6452–6453; b) M. Fujita, Y. Nakao, S. Matsunaga, M. Seiki, Y. Itoh, R. W. M. van Soest, N. Fusetani, *Tetrahedron* **2001**, *57*, 1229–1234; c) E. Ohta, S. Ohta, S. Ikegami, *Tetrahedron* **2001**, *57*, 4699–4703.
- [4] a) M. Ono, S. Sakuda, A. Suzuki, A. Isogai, *J. Antibiot.* **1997**, *50*, 111–118; b) M. Ono, S. Sakuda, H. Ikeda, K. Furihata, J.

- Nakayama, A. Suzuki, A. Isogai, *J. Antibiot.* **1998**, *51*, 1019–1028.
- [5] a) S. Matsunaga, N. Fusetani, Y. Kato, *J. Am. Chem. Soc.* **1991**, *113*, 9690–9692; b) E. W. Schmidt, M. K. Harper, D. J. Faulkner, *J. Nat. Prod.* **1997**, *60*, 779–782; c) D. Wolf, F. J. Schmitz, F. Qiu, M. Kelly-Borges, *J. Nat. Prod.* **1999**, *62*, 170–172; d) A. S. Ratnayake, R. A. Davis, M. K. Harper, C. A. Veltri, C. D. Andjelic, L. R. Barrows, C. M. Ireland, *J. Nat. Prod.* **2005**, *68*, 104–107; e) R. F. Angawi, G. Bavestrello, B. Calcinai, H. A. Dien, G. Donnarumma, M. A. Tufano, I. Paoletti, E. Grimaldi, G. Chianese, E. Fattorusso, O. Tagliatela-Scafati, *Mar. Drugs* **2011**, *9*, 2809–2817; f) N. U. Sata, S. Matsunaga, N. Fusetani, R. W. M. van Soest, *J. Nat. Prod.* **1999**, *62*, 969–971; g) R. Kumar, R. Subramani, K.-D. Feussner, W. Aalbersberg, *Mar. Drugs* **2012**, *10*, 200–208.
- [6] N. U. Sata, S. Wada, S. Matsunaga, S. Watabe, R. W. M. van Soest, N. Fusetani, *J. Org. Chem.* **1999**, *64*, 2331–2339.
- [7] a) S. V. Pronin, S. A. Kozmin, *J. Am. Chem. Soc.* **2010**, *132*, 14394–14396; b) J. J. Beiger, **2013**. Total Synthesis of Aflastatin A. Doctoral dissertation, Harvard University, <http://nrs.harvard.edu/urn-3:HUL.InstRepos:10974701>; c) S. Loscher, R. Schobert, *Chem. Eur. J.* **2013**, *19*, 10619–10624; d) A. Yajima, A. Kawajiri, A. Mori, R. Katsuta, T. Nukada, *Tetrahedron Lett.* **2014**, *55*, 4350–4354; e) M. L. Hofferberth, R. Brückner, *Angew. Chem. Int. Ed.* **2014**, *53*, 7328–7334; *Angew. Chem.* **2014**, *126*, 7456–7462; f) O. Hartmann, M. Kalesse, *Angew. Chem. Int. Ed.* **2014**, *53*, 7335–7338; *Angew. Chem.* **2014**, *126*, 7463–7466.
- [8] a) R. N. Lacey, *J. Chem. Soc.* **1954**, 832–839; b) R. N. Lacey, *J. Chem. Soc.* **1954**, 850–854.
- [9] L. T. Burke, D. J. Dixon, S. V. Ley, F. Rodríguez, *Org. Biomol. Chem.* **2005**, *3*, 274–280.
- [10] E. Biron, H. Kessler, *J. Org. Chem.* **2005**, *70*, 5183–5189.
- [11] Z. Huang, M. Zhang, S. D. Burton, L. N. Katsakhyan, H. Ji, *ACS Chem. Biol.* **2014**, *9*, 193–201.
- [12] a) B. K. Gorityala, J. Ma, K. K. Pasunooti, S. Cai, X.-W. Liu, *Green Chem.* **2011**, *13*, 573–577; b) T. K. M. Shing, H. M. Cheng, W. F. Wong, C. S. K. Kwong, J. Li, C. B. S. Lau, P. S. Leung, C. H. K. Cheng, *Org. Lett.* **2008**, *10*, 3145–3148.
- [13] F. Zhang, A. Vasella, *Carbohydr. Res.* **2007**, *342*, 2546–2556.
- [14] a) T. Fukuyama, C.-K. Jow, M. Cheung, *Tetrahedron Lett.* **1995**, *36*, 6373–6374; b) C. Huo, C. Wang, M. Zhao, S. Peng, *Chem. Res. Toxicol.* **2004**, *17*, 1112–1120.
- [15] N. A. Heaps, C. D. Poulter, *J. Org. Chem.* **2011**, *76*, 1838–1843.
- [16] X. Wei, R. J. K. Taylor, *Tetrahedron Lett.* **1998**, *39*, 3815–3818.
- [17] S. V. Ley, P. R. Woodward, *Tetrahedron Lett.* **1987**, *28*, 345–346.

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Supporting Information

Total Synthesis of Aurantioside G, an *N*- β -Glycosylated 3-Oligoenoyltetramic Acid from *Theonella swinhoei*

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S1

1. General information

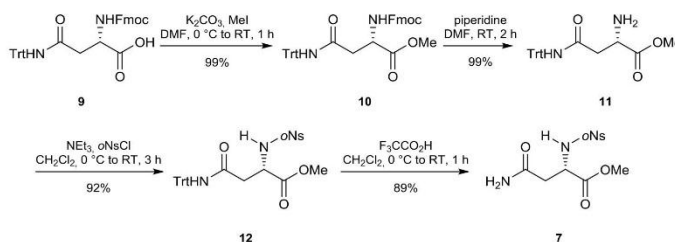
Nuclear Magnetic Resonance Spectroscopy: ^1H NMR and ^{13}C spectra were acquired on a Bruker Avance 300 (300 MHz) or on a Bruker Avance DRX-500 spectrometer (500 MHz, with cryoprobe) and were referenced to the signals of the solvents given as internal standards (δ in ppm: CDCl_3 7.26 for ^1H , 77.16 for ^{13}C ; $[\text{D}_4]$ MeOD 3.31 for ^1H , 49.00 for ^{13}C).^[1] The signal multiplicity was denoted as singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), and broad (br). Coupling constants (J) were measured in Hertz. The chemical shift of quaternary carbon atoms with low intensity was determined by 2D spectra (HMBC-experiments). *Mass Spectrometry:* High resolution mass spectra (HRMS) were recorded with a UPLC/Orbitrap MS system in ESI mode. *Infrared spectroscopy:* IR spectra were recorded with a Spectrum One FT-IR spectrometer (PerkinElmer) with an ATR unit. *Specific optical rotations* were recorded on a PerkinElmer 241 Polarimeter at 589 nm (Na-D line) using solutions in chloroform and methanol *p.A.*. Specific rotations ($[\alpha]_D$) are reported in units of $10^{-1} \text{ deg cm}^2 \text{ g}^{-1}$. *Melting points* of crystalline compounds were measured on a Büchi Melting Point H-565 apparatus and are not corrected. All reagents were purchased from commercial sources and were used without further purifications unless stated otherwise. Dry dichloromethane was obtained by heating under reflux over CaH_2 and subsequent distillation. Dry tetrahydrofuran was obtained by heating under reflux over sodium potassium alloy and subsequent distillation. Unless otherwise stated reactions were carried out under inert gas atmosphere in oven dried glass ware. *Chromatography:* Analytical HPLC was performed on a Beckmann system with solvent module 126 and a diode array detector 168 equipped with a Phenomenex Kinetex $5\mu\text{m}$ C-18 reversed phase column sized $250 \times 4.6 \text{ mm}$, pore size 100 \AA . Analytical thin layer chromatography (TLC) was carried out using Merck Kieselgel 60GF₂₅₄ pre-coated aluminium-backed plates. The compounds were visualized with UV light (254 nm and/or 360 nm) and/or ceric ammonium molybdate (CAM) and/or potassium permanganate. For column chromatography silica 60 (230-400 mesh) was used.

All compounds were stored under an atmosphere of argon with exclusion of light in a fridge at -20°C . All compounds with conjugated alkenes were handled and reacted under light exclusion.

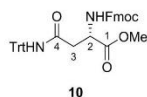
2. Experimental procedures

2.1. Synthesis of the amino acid 7

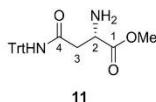
Overview of the synthesis of methyl (S)-2-((o-nitrobenzenesulfonyl)-amino)-4-oxo-amino-butanoate **7**



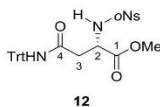
Methyl (S)-2-((9H-fluoren-9-yl)methoxycarbonyl-amino)-4-oxo-4-(tritylamino)butanoate **10**^[2]



To a stirred solution of (S)-2-((9H-fluoren-9-yl)-methoxycarbonylamino)-4-oxo-4-(tritylamino)butyric acid (**9**) (2.983 g, 5.00 mmol, 1.00 eq) in DMF (50 mL) at 0 °C was added K₂CO₃ (0.829 g, 6.00 mmol, 1.20 eq) and MeI (0.47 mL, 7.50 mmol, 1.50 eq). The solution was warmed to room temperature and stirred for another hour. Then aqueous Na₂S₂O₃ (10% wt., 50 mL) was added and the phases were separated. The aqueous phase was extracted twice with EtOAc (2x75 mL). The combined organic phases were washed twice with water (2x50 mL), once with brine (50 mL) and then dried (Na₂SO₄). The volatiles were evaporated to give **10** (3.021 g, 4.947 mmol, 99%) as a colorless solid of m.p. 189.0 °C; [α]_D²⁵=20.1 (c=1.00, CHCl₃); R_f 0.48 (1:1 c-hexane / EtOAc); ¹H NMR (500 MHz, CDCl₃): δ 7.80-7.73 (m, 2H; ArH), 7.63-7.57 (m, 2H; ArH), 7.44-7.36 (m, 2H; ArH), 7.34-7.24 (m, 10H; ArH), 7.21-7.11 (m, 7H; ArH), 6.74 (s, 1H; NH-Trityl), 6.11 (d, J=8.7 Hz, 1H; NH-Fmoc), 4.62 (dt, J=4.4, 8.7 Hz, 1H; 2-H), 4.42 (dd, J=7.5, 10.4 Hz, 1H; CH₂-Fmoc), 4.30 (dd, J=7.1, 10.4 Hz, 1H; CH₂-Fmoc), 4.22 (dd, J=7.1 Hz, 1H; CH-Fmoc), 3.69 (s, 3H; OMe), 3.11 (dd, J=4.4, 15.8 Hz, 1H, 3-H^a), 2.80 ppm (dd, J=3.9, 15.8 Hz, 1H; 3-H^b); ¹³C NMR (125 MHz, CDCl₃): δ 171.6, 169.4, 156.4, 144.4, 144.0, 143.9, 141.4, 128.7, 128.2, 127.8, 127.3, 127.2, 125.3, 120.1, 71.1, 67.3, 52.9, 51.0, 47.2, 38.8 ppm; IR ν[cm⁻¹]=3321 (w), 1747 (m), 1702 (m), 1657 (m), 1518 (m), 1448 (m), 1284 (m), 1219 (m), 1085 (w), 1038 (m), 768 (m), 741 (s), 701 (s), 624 (m), 580 (m). Data in accordance with literature.^[2]

Methyl (2S)-2-amino-4-oxo-4-(tritylamino)butanoate 11

A stirred solution of methyl ester **10** (3.021 g, 4.945 mmol, 1.00 eq) in DMF (45 mL) at room temperature was treated with piperidine (5 mL) and stirred for another hour. Sat. aqueous NH_4Cl was added, the solution was diluted with EtOAc (50 mL), and the phases were separated. The aqueous layer was extracted twice with EtOAc (2x75 mL), and the combined organic phases were washed twice with water (2x50 mL), once with brine (50mL), and dried (Na_2SO_4). The volatiles were removed in vacuo and the solid remainder was suspended in *c*-hexane and filtered off to give compound **11** (1.908 g, 4.912 mmol, 99%) as a colorless solid of m.p. 131.2 °C; $[\alpha]_D^{25}=8.8$ ($c=1.00$, CHCl_3); R_f 0.05 (1:1 *c*-hexane / EtOAc); ^1H NMR (500 MHz, CDCl_3): δ 8.45 (s, br, 1H; *NH*-Trityl), 7.33-7.19 (m, 15H; ArH), 3.84 (dd, $J=3.7$, 8.4 Hz, 1H; 2-H), 3.72 (s, 3H; OMe), 2.69 (dd, $J=3.7$, 15.7 Hz, 1H; 3- H^a), 2.58 (dd, $J=8.4$, 15.7 Hz, 1H; 3- H^b), 1.82 ppm (s, br., 2H; NH_2); ^{13}C NMR (125 MHz, CDCl_3): δ 174.8, 169.3, 144.9, 128.8, 128.0, 127.0, 63.2, 52.5, 51.5, 40.9 ppm; IR $\nu[\text{cm}^{-1}]=3231$ (w), 3026 (w), 1732 (m), 1639 (m), 1526 (m), 1491 (m), 1446 (m), 1196 (m), 1021 (m), 831 (m), 763 (m), 752 (m), 722 (w), 699 (s), 638 (m), 624 (m), 578 (m). Data in accordance with literature.^[2]

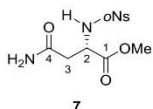
Methyl (2S)-2-(*o*-nitrobenzenesulfonylamino)-4-oxo-4-(tritylamino)butanoate 12^[3]

A stirred solution of amine **11** (1.908 g, 4.912 mmol, 1.00 eq) in dry CH_2Cl_2 (50 mL) at 0 °C was treated with NEt_3 (1.00 mL, 7.368 mmol, 1.50 eq) and oNsCl (1.197 g, 5.403 mmol, 1.10 eq). The mixture was warmed to room temperature and stirred for 16 h. Then aqueous NaHCO_3 (10% wt., 50 mL) was added and the phases were separated. The aqueous phase was extracted twice with CH_2Cl_2 (2x75 mL), the combined organic phases were dried (Na_2SO_4), the solvent evaporated, and the remaining yellow oil was purified by column chromatography on silica gel (25% EtOAc in *c*-hexane) to give compound **12** (2.602 g, 4.536 mmol, 92%) as a colorless solid of m.p. 90-94 °C (decomp.); $[\alpha]_D^{25}=88.9$ ($c=1.00$, CHCl_3); R_f 0.37 (1:1 *c*-hexane / EtOAc); ^1H NMR (500 MHz, CDCl_3): δ 8.09-8.01 (m, 1H; ArH-Nosyl), 7.89-7.82 (m, 1H; ArH-Nosyl), 7.72-7.65 (m, 2H; ArH-Nosyl), 7.36-7.11 (m, 15H; ArH), 6.67 (s, 1H; *NH*-Trityl), 6.62 (d, $J=8.7$ Hz, 1H; *NH*-Nosyl), 4.42 (dt, $J=4.2$, 8.7 Hz, 1H; 2-H), 3.42 (s, 3H; OMe), 3.14 (dd, $J=4.2$, 16.0 Hz, 1H; 3- H^a), 2.90 ppm (dd, $J=4.6$, 16.0 Hz, 1H; 3- H^b); ^{13}C NMR (125 MHz, CDCl_3): δ 170.6, 168.5, 147.8, 144.3, 134.6, 133.5, 130.6, 128.8, 128.2, 127.3, 125.4, 71.3, 53.6, 52.9,

S4

40.3 ppm; IR $\nu[\text{cm}^{-1}]$ =3321 (w), 3026 (w), 1742 (m), 1662 (m), 1595 (w), 1539 (m), 1491 (m), 1445 (m), 1411 (m), 1351 (m), 1274 (m), 1214 (m), 1165 (s), 1120 (m), 1036 (m), 904 (w), 853 (m), 742 (m), 698 (s), 655 (m), 624 (m), 591 (s), 556 (m). HRMS (ES⁺) m/z [M+H] calcd for C₃₀H₂₇N₃O₇Na⁺ 596.14619, found 596.14594.

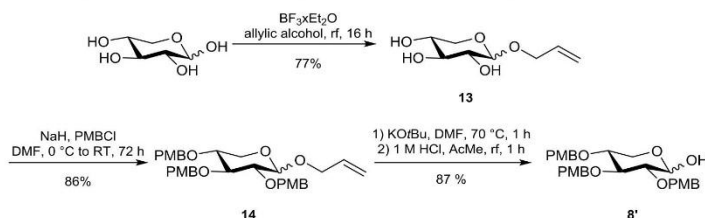
Methyl (2S)-2-(*o*-nitrobenzenesulfonylamino)-4-oxo-4-aminobutanoate **7**



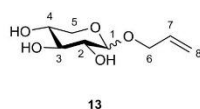
A stirred solution of **12** (2.602 g, 4.536 mmol, 1.00 eq) in dry CH₂Cl₂ (10 mL) at 0 °C was treated with trifluoroacetic acid (10 mL, 130.679 mmol, 28.80 eq), warmed to room temperature, and stirred for 1 h. The mixture was diluted with toluene (20 mL) and evaporated five times to remove the acid azeotropically. The yellow oily remainder was purified by column chromatography (silica, 5% methanol in CH₂Cl₂) to afford compound **7** (1.338 g, 4.039 mmol, 89%) as a colorless solid of m.p. 80.2 °C; $[\alpha]_D^{25}$ =−166.5 (c=1.00, CHCl₃); R_f 0.13 (1:3 *c*-hexane / EtOAc); ¹H NMR (300 MHz, CDCl₃): δ 8.14–8.06 (m, 1H; ArH-Nosyl), 7.94–7.86 (m, 1H; ArH-Nosyl), 7.77–7.69 (m, 2H; ArH-Nosyl), 7.08 (d, *J*=9.0 Hz, 1H; *NH*-Nosyl), 6.03 (d, br., *J*=29.1 Hz, 2H; NH₂), 4.45 (dt, *J*=4.9, 9.0 Hz, 1H; 2-H), 3.51 (s, 3H; OMe), 3.00 (dd, *J*=4.9, 16.3 Hz, 1H; 3-H^a), 2.82 ppm (dd, *J*=4.6, 16.3 Hz, 1H; 3-H^b); ¹³C NMR (75 MHz, CDCl₃): δ 171.9, 170.9, 147.7, 134.7, 133.6, 133.0, 130.6, 125.4, 53.4, 52.9, 38.2 ppm; IR $\nu[\text{cm}^{-1}]$ =3473 (w), 3353 (w), 3200 (w), 1741 (m), 1669 (m), 1611 (w), 1538 (s), 1440 (w), 1408 (m), 1348 (s), 1303 (m), 1261 (m), 1217 (m), 1164 (s), 1119 (m), 1059 (m), 1042 (w), 971 (w), 911 (w), 854 (m), 783 (m), 729 (s), 702 (m), 654 (m); HRMS (ES⁺) m/z [M+H] calcd for C₁₁H₁₃N₃O₇Na⁺ 354.03664, found 354.03591.

2.2. Synthesis of the *N*-D-xylosylasparaginate 3'

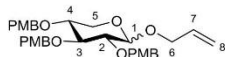
Overview of the synthesis of 2,3,4-tri-*O*-(4-methoxybenzyl)-D-xylopyranoside 8'



1-*O*-Allyl-D-xylopyranoside 13^[4]



A stirred suspension of D-xylose (6.005 g, 40.00 mmol, 1.00 eq) in allylic alcohol (80 mL) at room temperature was treated with $\text{BF}_3 \cdot \text{Et}_2\text{O}$ (1.23 mL, 10.00 mmol, 0.25 eq) and then heated under reflux for 16 h. The solution was cooled to room temperature and the solvent was evaporated to leave a brown solid, which was purified by column chromatography (silica, 5% methanol in CH_2Cl_2) to afford compound **13** (5.887 g, 30.953 mmol, 77%, $\alpha/\beta = 2.72/1.00$) as a light brownish solid of m.p. 76.4 °C; $[\alpha]_{\text{D}}^{25} = 114.1$ ($c = 1.00$, CHCl_3); R_f 0.23 (9:1 CH_2Cl_2 / MeOH); α -epimer: ^1H NMR (300 MHz, CDCl_3): δ 6.00–5.83 (m, 1H; 7-H), 5.36–5.26 (m, 1H; 8- H^a), 5.25–5.16 (m, 1H; 8- H^b), 4.82 (d, $J = 3.7$ Hz, 1H; 1-H), 4.63 (s, br., 3H; OH), 4.19 (ddt, $J = 1.2, 5.4, 12.9$ Hz, 1H; 6- H^a), 4.01 (ddt, $J = 1.2, 6.4, 12.9$ Hz, 1H; 6- H^b), 3.76–3.67 (m, 1H; 3-H), 3.67–3.59 (m, 1H; 5- H^a), 3.63–3.56 (m, 1H; 4-H), 3.54–3.36 ppm (m, 2H, 2-H; 5- H^b); ^{13}C NMR (75 MHz, CDCl_3): δ 133.9, 118.2, 97.7, 74.7, 72.1, 70.1, 68.5, 61.9 ppm; β -epimer: ^1H NMR (300 MHz, CDCl_3): δ 6.00–5.83 (m, 1H; 7-H), 5.36–5.26 (m, 1H; 8- H^a), 5.25–5.16 (m, 1H; 8- H^b), 4.31 (ddt, $J = 1.3, 5.3, 12.8$ Hz, 1H; 6- H^a), 4.29 (d, $J = 7.2$ Hz, 1H; 1-H), 4.10 (ddt, $J = 1.1, 6.4, 12.8$ Hz, 1H; 6- H^b), 3.95 (dd, $J = 5.1, 11.3$ Hz, 1H; 5- H^a), 3.77–3.55 (m, 1H; 3-H, 4-H), 3.44–3.36 (m, 1H; 2-H), 3.22 ppm (dd, $J = 9.9, 11.3$ Hz, 1H; 5- H^b); ^{13}C NMR (75 MHz, CDCl_3): δ 133.9, 118.3, 102.3, 76.3, 73.2, 70.3, 69.7, 65.5 ppm; IR $\nu[\text{cm}^{-1}] = 753$ (w), 896 (w), 931 (s), 993 (s), 1016 (s), 1026 (s), 1076 (w), 1095 (m), 1125 (m), 1140 (m), 1217 (w), 1243 (w), 1262 (w), 1296 (w), 1342 (w), 1379 (w), 1392 (w), 1434 (w), 2882 (w), 2900 (w), 2940 (w), 2984 (w), 3107 (w), 3203 (w), 3409 (w). Data in accordance with literature.^[4]

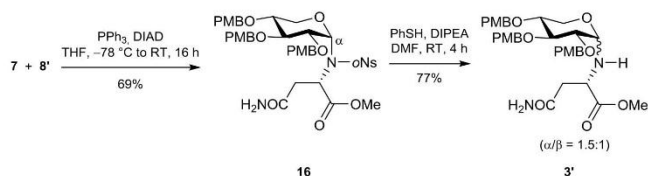
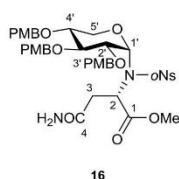
1-O-Allyl-2,3,4-tri-O-(4-methoxybenzyl)-D-xylopyranoside 14

14

A stirred solution of **13** (4.883 g, 25.673 mmol, 1.00 eq) in DMF (200 mL) at 0 °C was slowly treated with a suspension of 60% NaH in mineral oil (4.621g, 115.529 mmol, 4.50 eq). After 30 min, PMBCl (18.093 g, 115.529 mmol, 4.50 eq) was added and the resulting mixture was stirred for 72 h at room temperature. Then methanol (5.3 mL, 131.664 mmol, 5.13 eq) was added at 0 °C and stirring continued for another hour. The mixture was warmed to room temperature, diluted with EtOAc (300 mL), and washed with water (200 mL). The aqueous phase was extracted twice with EtOAc (2x200 mL). The combined organic phases were washed with sat. aqueous NaHCO₃ (200 mL) and brine (200 mL), and then dried (Na₂SO₄). The volatiles were removed in vacuo and the yellow oily residue was purified by column chromatography (silica, 10% EtOAc in *c*-hexane) to furnish compound **14** (12.114 g, 21.999 mmol, 86%, $\alpha/\beta = 1.48/1.00$) as a colorless foam; $[\alpha]_D^{25} = 20.2$ ($c = 1.00$, CHCl₃); R_f 0.39 (3:1 *c*-hexane / EtOAc); α -epimer: ¹H NMR (300 MHz, CDCl₃): δ 7.34-7.20 (m, 6H; ArH), 6.91-6.83 (m, 6H; ArH), 6.04-5.86 (m, 1H; 7-H), 5.34 (ddq, $J = 1.6, 7.2, 17.1$ Hz, 1H; 8-H^a), 5.25-5.18 (m, 1H; 8-H^b), 4.88-4.51 (m, 7H; CH₂-PMB, 1-H), 4.20-4.08 (m, 1H; 6-H^a), 3.98 (ddt, $J = 1.3, 6.6, 13.0$ Hz, 1H; 6-H^b), 3.93-3.84 (m, 1H; 3-H), 3.79 (s, 9H; OMe), 3.62-3.47 (m, 3H; 4-H, 5-H), 3.43 ppm (dd, $J = 3.6, 9.7$ Hz, 1H; 2-H); ¹³C NMR (75 MHz, CDCl₃): δ 159.5, 159.4, 133.9, 131.3, 130.6, 130.5, 129.7, 129.6, 129.4, 118.0, 113.9, 113.8, 95.9, 81.2, 79.4, 77.9, 75.5, 73.3, 73.0, 68.0, 60.1, 55.3 ppm; β -epimer: ¹H NMR (300 MHz, CDCl₃): δ 7.35-7.18 (m, 6H; ArH), 6.93-6.82 (m, 6H; ArH), 6.04-5.86 (m, 1H; 7-H), 5.34 (ddq, $J = 1.6, 7.2, 17.1$ Hz, 1H; 8-H^a), 5.25-5.18 (m, 1H; 8-H^b), 4.88-4.51 (m, 6H; CH₂-PMB), 4.37 (d, $J = 7.7$ Hz, 1H; 1-H), 4.37 (ddt, $J = 1.6, 5.3, 13.0$ Hz, 1H; 6-H^a), 4.08-4.20 (m, 1H; 6-H^b), 3.93-3.84 (m, 1H; 5-H^a), 3.80 (s, 9H; OMe), 3.62-3.47 (m, 2H; 3-H, 4-H), 3.37 (dd, $J = 7.7$ Hz, 1H; 2-H), 3.17 ppm (dd, $J = 9.9, 11.4$ Hz, 1H; 5-H^b); ¹³C NMR (75 MHz, CDCl₃): δ 159.3, 134.1, 131.0, 130.8, 130.4, 129.8, 129.6, 129.5, 117.3, 113.9, 113.8, 103.3, 83.5, 81.7, 77.6, 75.3, 74.7, 73.1, 70.2, 64.0, 55.3 ppm; IR ν [cm⁻¹]=2909 (w), 1612 (m), 1586 (w), 1464 (w), 1440 (w), 1359 (w), 1245 (s), 1172 (m), 1072 (s), 1033 (s), 908 (m), 820 (m), 728 (s); HRMS (ES⁺) m/z [M+Na] calcd for C₃₂H₃₈O₈Na⁺ 573.24589 found 573.24487.

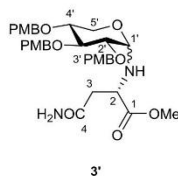
2,3,4-Tri-O-(4-methoxybenzyl)-D-xylopyranoside 8'**8'**

A stirred solution of **14** (2.212 g, 4.017 mmol, 1.00 eq) in DMF (40 mL) at room temperature was treated with KO^tBu (0.902g, 8.034 mmol, 2.00 eq), heated at 70 °C for 1 h, then chilled to room temperature, and finally neutralized with 1 M HCl. The mixture was extracted three times with EtOAc (3×60 mL), the combined organic phases were washed twice with water (2×60 mL), once with brine (60 mL), and then dried (Na₂SO₄). The solvent was evaporated to leave a yellow oil which was used without further purification. It was dissolved in acetone (36 mL), 1 M HCl (4 mL) was added at room temperature, and the resulting mixture was heated at reflux for 1 h. The solution was cooled to room temperature, diluted with EtOAc and washed with water (200 mL). The aqueous phase was extracted three times with EtOAc (3×150 mL), the combined organic phases were dried (Na₂SO₄) and concentrated to leave a pale yellow solid which was purified by column chromatography (silica, 25% EtOAc in *c*-hexane) to give pure **8'** (1.783 g, 3.492 mmol, 87%, $\alpha/\beta = 1.72/1.00$) as a colorless foam; $[\alpha]_D^{25} = 11.5$ ($c = 1.00$, CHCl₃); R_f 0.40 (1:1 *c*-hexane / EtOAc); α -epimer: ¹H NMR (300 MHz, CDCl₃): δ 7.31-7.21 (m, 6H; ArH), 6.90-6.82 (m, 6H; ArH), 5.05 (d, $J = 3.6$ Hz, 1H; 1-H), 4.82-4.52 (m, 6H; CH₂-PMB), 3.81 (s, 6H; OMe), 3.80 (s, 3H; OMe), 3.83-3.79 (m, 1H; 4-H), 3.79-3.72 (m, 1H; 5-H^a), 3.65-3.58 (m, 1H; 5-H^b), 3.58-3.47 (m, 1H; 3-H), 3.44 (dd, $J = 3.6, 8.9$ Hz, 1H; 2-H), 3.00 ppm (s, br., 1H; OH); ¹³C NMR (75 MHz, CDCl₃): δ 159.5, 159.4, 131.0, 130.5, 130.1, 129.8, 129.5, 114.0, 113.9, 91.7, 80.3, 79.3, 77.3, 75.3, 73.2, 73.0, 60.6, 55.4 ppm; β -epimer: ¹H NMR (300 MHz, CDCl₃): δ 7.31-7.21 (m, 6H; ArH), 6.90-6.82 (m, 6H; ArH), 4.82-4.52 (m, 7H; 1-H, CH₂-PMB), 3.94-3.86 (m, 1H; 5-H^a), 3.81 (s, 3H; OMe), 3.80 (s, 6H; OMe), 3.58-3.47 (m, 2H; 3-H, 4-H), 3.30-3.21 ppm (m, 2H; 2-H, 5-H^b); ¹³C NMR (75 MHz, CDCl₃): δ 159.6, 159.5, 130.9, 130.7, 130.4, 129.9, 129.7, 129.6, 114.0, 113.9, 97.9, 83.0, 82.1, 77.4, 75.2, 74.5, 73.1, 63.9, 55.4 ppm; IR ν [cm⁻¹]=3369 (br. w), 2908 (w), 2838 (w), 1614 (m), 1586 (w), 1514 (s), 1464 (w), 1440 (w), 1425 (w), 1398 (w), 1360 (w), 1301 (m), 1250 (s), 1171 (m), 1106 (m), 1076 (s), 1033 (s), 995 (m), 966 (m), 934 (m), 903 (w), 849 (w), 817 (s), 775 (w), 759 (w), 744 (w), 714 (w), 666 (w); HRMS (ES⁺) m/z [M+Na] calcd for C₂₉H₃₄O₈Na⁺ 533.21459 found 533.21576.

Overview of the synthesis of *N*-D-xylosylasparaginate **3'**

Methyl (2*S*)-*N*-(2',3',4'-tri-*O*-(4-methoxybenzyl)- α -D-xylopyranosyl)-2-(*o*-nitrobenzenesulfonylamino)-4-oxo-4-aminobutanoate **16**


A stirred solution of ester **7** (0.377 g, 1.138 mmol, 1.00 eq), saccharide **8'** (1.162 g, 2.276 mmol, 2.00 eq), and PPh_3 (0.597 g, 2.276 mmol, 2.00 eq) in dry THF (12 mL) at -78°C was treated with DIAD (0.45 mL, 2.276 mmol, 2.00 eq), allowed to warm to room temperature over 16 h, and then stripped of all volatiles. The yellow oily remainder was purified by column chromatography, eluting with 50% EtOAc in *c*-hexane, to afford compound **16** (0.649 g, 0.788 mmol, 69%) as a colorless foam; $[\alpha]_{\text{D}}^{25} = 1.7$ ($c = 1.00$, CHCl_3); R_f 0.31 (1:3 *c*-hexane / EtOAc); ^1H NMR (300 MHz, CDCl_3): δ 8.16 (d, $J = 7.6$ Hz, 1H; NH-Nosyl), 7.72–7.57 (m, 2H; ArH-Nosyl), 7.53–7.35 (m, 2H; ArH-Nosyl), 7.24–7.12 (m, 6H; ArH-PMB), 6.88–6.73 (m, 6H; ArH-PMB), 5.74 (s, br., 1H; NH_2), 4.97–4.83 (m, 2H; 2-H, CH_2 -PMB), 4.75–4.47 (m, 5H; CH_2 -PMB, 1'-H), 4.02 (dd, $J = 5.3, 11.1$ Hz, 1H; 5'-H^a), 4.10–4.00 (m, 1H; 3'-H), 3.73 (s, 3H; OMe-PMB), 3.70 (s, 3H; OMe-PMB), 3.72 (s, 3H; OMe-PMB), 3.72–3.66 (m, 1H; 3'-H), 3.64–3.56 (m, 2H; 2'-H, 4'-H), 3.37 (s, 3H; OMe), 3.25 (dd, $J = 11.1$ Hz, 1H; 5'-H^b), 3.11–2.91 (m, 1H; 3'-H^a), 2.58 ppm (dd, $J = 3.2, 16.2$ Hz, 1H; 3'-H^b); ^{13}C NMR (75 MHz, CDCl_3): δ 171.5, 169.7, 159.2, 159.0, 148.5, 133.6, 133.0, 132.8, 131.9, 131.8, 131.7, 131.6, 130.5, 130.3, 130.1, 129.3, 123.4, 113.7, 113.6, 87.6, 85.4, 78.1, 77.6, 77.4, 74.8, 73.8, 72.8, 66.4, 55.1, 52.2, 38.1 ppm; IR $\nu[\text{cm}^{-1}] = 2955$ (w), 2837 (w), 1740 (m), 1683 (m), 1612 (m), 1586 (w), 1544 (m), 1513 (s), 1464 (m), 1439 (m), 1407 (w), 1357 (m), 1302 (m), 1246 (s), 1172 (s), 1075 (s), 1033 (s), 907 (s), 851 (m), 822 (m), 726 (s), 763 (w); HRMS (ES^+) m/z $[\text{M} + \text{H}]$ calcd for $\text{C}_{40}\text{H}_{46}\text{N}_3\text{O}_{14}\text{S}^+$ 824.26950 found 824.26988.

Methyl (2S)-N-(2',3',4'-tri-O-(4-methoxybenzyl)-D-xylopyranosyl)-2-amino-4-oxo-4-aminobutanoate 3'

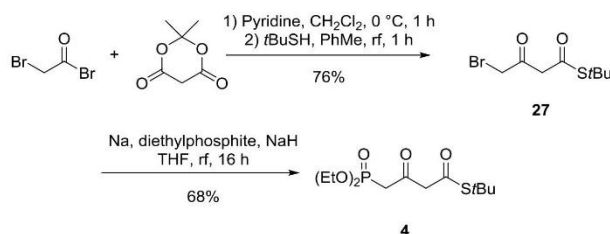


A stirred solution of *N*-glycoside **16** (0.358 g, 0.435 mmol, 1.00 eq) in DMF (5 mL) at room temperature was treated with DIPEA (0.61 mL, 3.476 mmol, 8.00 eq) and PhSH (0.45 mL, 4.345 mmol, 10.00 eq) and stirred for a further 4 h at this temperature. The mixture was diluted with EtOAc and water, the aqueous phase was extracted twice with EtOAc (2×25 mL), and the combined organic phases were washed twice with water (2×25 mL) and once with brine (25 mL), and then dried (Na₂SO₄). The solvent was evaporated and the yellowish oily residue was purified by column chromatography (silica, 25% *c*-hexane in EtOAc) to give **3'** (0.214 g, 0.335 mmol, 77%, $\alpha/\beta = 1.45/1.00$) as a colorless foam; $[\alpha]_D^{25} = -14.0^\circ$ ($c=1.00$, CHCl₃); R_f 0.37 (EtOAc); α -epimer: ¹H NMR (500 MHz, CDCl₃): δ 7.32–7.18 (m, 6H; ArH), 6.92–6.81 (m, 6H; ArH), 6.79 (d, br., 1H; NH₂), 5.44 (d, br., 1H; NH₂), 4.66–4.59 (m, 2H; CH₂-PMB), 4.59–4.45 (m, 4H; CH₂-PMB), 4.44 (d, br., $J=4.0$ Hz, 1H; 1'-H), 3.87–3.82 (m, 1H; 2-H), 3.81 (s, 3H; OMe-PMB), 3.80 (s, 6H; OMe-PMB), 3.71 (s, 3H; OMe), 3.70–3.63 (m, 2H; 4'-H, 5'-H^a), 3.60–3.51 (m, 1H; 5'-H^b), 3.43 (dd, $J=4.0, 7.2$ Hz, 1H; 2'-H), 3.41–3.36 (m, 1H; 3'-H), 2.55–2.48 ppm (m, 2H; 3-H); ¹³C NMR (125 MHz, CDCl₃): δ 173.7, 172.8, 159.5, 159.4, 159.3, 130.5, 130.4, 130.0, 129.9, 129.6, 129.5, 114.0, 113.9, 83.4, 77.7, 77.2, 75.1, 74.1, 72.8, 72.3, 61.5, 55.2, 54.6, 37.3 ppm; β -epimer: ¹H NMR (500 MHz, CDCl₃): δ 7.32–7.18 (m, 6H; ArH), 6.92–6.81 (m, 6H; ArH), 6.69 (s, br., 1H; NH₂), 5.55 (s, br., 1H; NH₂), 4.86 (d, $J=11.0$ Hz, 1H; CH₂-PMB), 4.83 (d, $J=11.3$ Hz, 1H; CH₂-PMB), 4.78 (d, $J=10.6$ Hz, 1H; CH₂-PMB), 4.68 (d, $J=11.0$ Hz, 1H; CH₂-PMB), 4.66–4.59 (m, 2H; CH₂-PMB), 3.85 (d, $J=8.3$ Hz, 1H; 1'-H), 3.85–3.79 (m, 2H; 2-H, 5'-H^a), 3.80 (s, 6H; OMe-PMB), 3.79 (s, 3H; OMe-PMB), 3.72 (s, 3H; OMe), 3.60–3.51 (m, 2H; 3'-H, 4'-H), 3.17 (dd, $J=8.3, 8.6$ Hz, 1H; 2'-H), 3.11 (dd, $J=10.4, 10.7$ Hz, 1H; 5'-H^b), 2.58 (dd, $J=4.0, 15.7$ Hz, 1H; 3-H^a), 2.42 ppm (dd, $J=7.9, 15.7$ Hz, 1H; 3-H^b); ¹³C NMR (125 MHz, CDCl₃): δ 173.5, 172.6, 159.5, 159.4, 159.3, 130.8, 130.3, 130.1, 129.7, 129.5, 113.9, 89.6, 84.9, 80.3, 78.1, 75.3, 74.7, 73.0, 65.1, 55.3, 52.5, 38.9 ppm; IR ν [cm⁻¹]=3409 (w), 3330 (w), 3228 (w), 2900 (w), 1732 (s), 1650(w), 1635 (m), 1613 (s), 1586 (w), 1513 (s), 1458 (m), 1437 (w), 1353 (w), 1302 (w), 1247 (s), 1172 (s), 1107 (m), 1064 (s), 1032 (s), 972 (m), 937 (m), 895 (w), 815 (m), 764 (w), 719 (m), 632 (m), 612 (m), 652 (m); HRMS (ES⁺) m/z [M+H] calcd for C₃₄H₄₃N₂O₁₀ 639.29122, found 639.29077.

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2.3. Synthesis the sidechain 2

Overview of the synthesis of S-*tert*-butyl-4-(diethoxyphosphono)-3-oxobutanethioate **4**



S-*tert*-Butyl 4-bromo-3-oxo-butanethioate **27**



27 was synthesized according to a literature protocol,^[5,6] starting from 2-bromoacetyl bromide and Meldrum's acid. The obtained spectra were in accordance with the literature. In CDCl_3 the product exists as a tautomeric mixture of keto and enol forms in a ratio of 3.12:1.00; R_f 0.66 (4:1 *c*-hexane / EtOAc); ^1H NMR (300 MHz, CDCl_3): δ 12.68 (s, 1H; OH^e), 5.56 (s, 1H; 2-H^e), 4.03 (s, 2H; 2-H^k), 3.81 (s, 2H; 4-H^e), 3.77 (s, 2H; 4-H^k), 1.49 (s, 9H; *t*Bu^e), 1.46 ppm (s, 9H; *t*Bu^k); ^{13}C NMR (75 MHz, CDCl_3) δ 196.9, 194.0, 192.2, 167.5, 101.2, 59.3, 55.1, 49.5, 48.9, 40.0, 34.4, 30.1, 29.7, 28.8 ppm; HRMS (ES^+): not detectable.

S-*tert*-Butyl 4-(diethoxyphosphono)-3-oxobutanethioate **4**

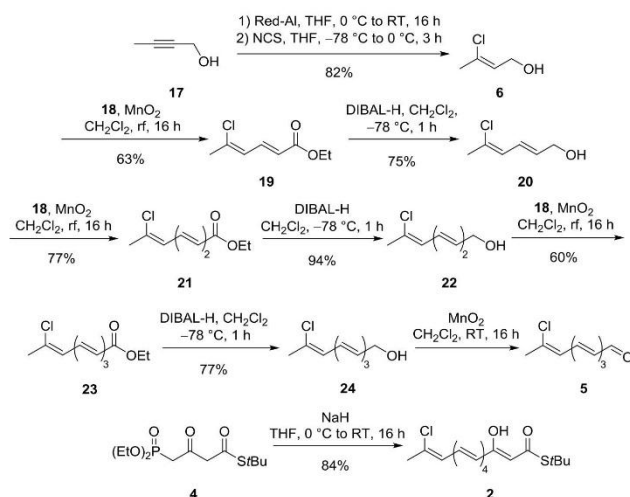


Phosphonate **4** was synthesized according to literature protocol,^[5,6] The obtained spectra were in accordance with the literature. In CDCl_3 the product exists as a tautomeric mixture of keto and enol forms in a ratio of 3.50:1.00; R_f 0.28 (1:3 *c*-hexane / EtOAc); ^1H NMR (300 MHz, CDCl_3): δ 12.93 (s, br., 1H; OH), 5.44 (d, $J=3.2$ Hz, 1H; 2-H^e), 4.18-4.07 (m, 8H; CH_2CH_3), 3.77 (s, 2H; 2-H^k), 3.22 (d, $J=22.8$ Hz, 2H; 4-H^k), 2.69 (d, $J=22.1$ Hz, 2H; 4-H^e), 1.47 (s, 9H; *t*Bu^e), 1.44 (s, 9H; *t*Bu^k), 1.31 ppm (dt, $J=0.4, 7.1$ Hz, 12H; CH_3CH_2); ^{13}C NMR (75 MHz, CDCl_3): δ

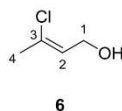
S11

194.2, 194.1, 192.3, 102.3, 102.2, 62.8, 62.7, 58.6, 49.3, 43.6, 41.9, 34.7, 32.9, 30.2, 29.7, 16.4, 16.3 ppm; HRMS (ES⁺): not detectable.

Overview of the synthesis of (2*Z*,4*E*,6*E*,8*E*,10*E*,12*Z*) *S*-*tert*-butyl 13-chloro-3-hydroxy-tetradeca-2,4,6,8,10,12-hexaenethioate **2**



(*Z*) 3-chloro-but-2-en-1-ol **6**^[7]

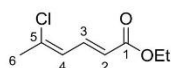


A stirred solution of 2-butyne-1-ol (**18**) (1.50 mL, 20.0 mmol, 1.00 eq) in dry THF (80 mL) at 0 °C was treated dropwise via a syringe with a Red-Al solution (70% wt. in toluene; 9.50 mL, 34.0 mmol, 1.70 eq) and allowed to warm slowly to room temperature over 16 h. The mixture was cooled to –78 °C and a suspension of NCS (5.074 g, 38.00 mmol, 1.90 eq) in dry THF (40 mL) was slowly added. The reaction was stirred at –78 °C for an additional hour and then for 2 h at 0 °C. Aqueous citric acid (33% wt., 100 mL) was added at 0 °C, the aqueous layer of the mixture was separated and extracted three times with diethyl ether (3×100 mL). The combined organic phases were washed with brine (200 mL) and dried (Na₂SO₄). The solution was concentrated carefully (max 35 °C, 350 mbar) to give a yellow oil which was purified by column chromatography on silica gel eluting with a gradient of 10% to 40% diethyl ether in *n*-pentane to afford compound **6** (1.748 g, 16.405 mmol, 82%) as a colorless oil. The solvent

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was evaporated carefully because of the fugacity of the compound. R_f 0.49 (1:1 *c*-hexane / EtOAc); ^1H NMR (300 MHz, CDCl_3): δ 5.67 (dt, $J=1.2, 6.3$ Hz, 1H; 2-H), 4.22 (dq, $J=1.2, 6.3$ Hz, 2H; 1-H), 2.58 (s, br., 1H; OH), 2.09 ppm (q, $J=1.2$ Hz, 3H; 4-H); ^{13}C NMR (75 MHz, CDCl_3): δ 132.6, 125.1, 59.7, 26.2 ppm; IR $\nu[\text{cm}^{-1}]$ =1686 (s), 1634 (w), 1435 (w), 1267 (w), 1205 (w), 1158 (m), 1095 (m), 1008 (w), 971(m), 880 (w); HRMS $[\text{ES}^+]$: not detectable; data in accordance with the literature.^[7]

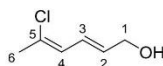
Ethyl (2*E*,4*Z*)-5-chloro-hexa-2,4-dienoate **19**



19

A stirred solution of alcohol **6** (1.748 g, 16.405 mmol, 1.00 eq) in dry CH_2Cl_2 (160 mL) at room temperature was treated with ylide **18** (6.858 g, 19.687 mmol, 1.20 eq) and MnO_2 (14.259 g, 164.010 mmol, 10.00 eq) and then heated at reflux for 16 h. The mixture was allowed to cool to room temperature and the resulting suspension was filtered through a short plug of celite. The solvent was evaporated to leave a red oil which was purified by column chromatography eluting with 2% EtOAc in *c*-hexane to afford compound **19** (1.805 g, 10.337 mmol, 63%) as a colorless oil; R_f 0.88 (2:1 *c*-hexane / EtOAc); ^1H NMR (300 MHz, CDCl_3): δ 7.59 (dd, $J=10.8, 15.5$ Hz, 1H; 3-H), 6.22 (ddq, $J=0.8, 1.2, 10.8$ Hz, 1H; 2-H), 5.89 (d, $J=15.5$ Hz, 1H; 4-H), 4.22 (q, $J=7.2$ Hz, 2H; CH_2CH_3), 2.45 (s, 3H; 6-H), 1.30 ppm (t, $J=7.2$ Hz, 3H; CH_3CH_2); ^{13}C NMR (75 MHz, CDCl_3): δ 185.2, 140.2, 139.4, 123.8, 122.3, 60.6, 27.2, 14.4 ppm; IR $\nu[\text{cm}^{-1}]$ = 2980 (w), 1709 (s), 1636 (s), 1616 (w), 1444 (w), 1367 (m), 1316 (s), 1253 (s), 1188 (s), 1139 (s), 1094 (s), 1035 (m), 931 (m), 878 (m), 750 (w), 718 (m), 630 (m), 577 (m); HRMS (ES^+) m/z $[\text{M}+\text{H}]$ calcd for $\text{C}_8\text{H}_{12}\text{ClO}_2^+$ 175.05203, found 175.05193.

(2*E*,4*Z*)-5-Chloro-hexa-2,4-dien-1-ol **20**

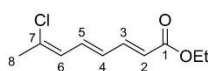


20

A stirred solution of ester **19** (1.805 g, 10.337 mmol, 1.00 eq) in dry CH_2Cl_2 (70 mL) at -78°C was treated with a 1 M DIBAL-H solution in *n*-hexane (22.7 mL, 22.741 mmol, 2.20 eq) and stirred for 1 h at this temperature. The mixture was allowed to warm to room temperature and aqueous citric acid (33% wt., 75 mL) was added. The phases were separated and the aqueous phase was extracted twice with CH_2Cl_2 (2×50 mL). The combined organic phases were dried (Na_2SO_4) and the solvent was evaporated to give a colorless oil which was purified by column

chromatography eluting with 15% EtOAc in *c*-hexane affording compound **20** (1.023 g, 7.716 mmol, 75%) as a colorless oil; R_f 0.33 (2:1 *c*-hexane / EtOAc); ^1H NMR (300 MHz, CDCl_3): δ 6.54 (ddt, $J=1.3, 10.2, 15.4$ Hz, 1H; 3-H), 6.07 (d, $J=10.2$ Hz, 1H; 4-H), 5.86 (dt, $J=5.7, 15.4$ Hz, 1H; 2-H), 4.19 (d, $J=5.7$ Hz, 2H; 1-H), 2.15 (s, 3H; 6-H), 1.78-1.89 ppm (m, 1H; OH); ^{13}C NMR (75 MHz, CDCl_3): δ 133.1, 131.9, 126.8, 124.6, 63.4, 26.5 ppm; IR $\nu[\text{cm}^{-1}]$ =3286 (br. w), 2917 (w), 2827 (w), 1656 (w), 1625 (w), 1425 (w), 1380 (w), 1311 (w), 1249 (w), 1104 (m), 1073 (m), 967 (s), 914 (m), 862 (m), 683 (m), 646 (s), 613 (m), 564 (m); HRMS (ES^+) m/z [M-OH] calcd for $\text{C}_6\text{H}_8\text{Cl}^+$ 115.03090, found 115.03090.

Ethyl (2*E*,4*E*,6*Z*)-7-chloro-octa-2,4,6-trienoate **21**

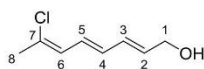


21

A stirred solution of alcohol **20** (1.023 g, 7.716 mmol, 1.00 eq) in dry CH_2Cl_2 (80 mL) at room temperature was treated with ylide **18** (3.226 g, 9.259 mmol, 1.20 eq) and MnO_2 (6.708 g, 77.155 mmol, 10.00 eq) and the mixture was heated under reflux for 16 h. It was allowed to cool to room temperature and the resulting suspension was filtered through a short plug of celite. The solvent was evaporated to leave a red oil which was purified by column chromatography eluting with 5% EtOAc in *c*-hexane to give **21** (1.192 g, 5.940 mmol, 77 %) as a colorless solid of m.p. 40.2 °C; R_f 0.60 (3:1 *c*-hexane / EtOAc); ^1H NMR (500 MHz, CDCl_3): δ 7.30 (dd, $J=11.4, 15.3$ Hz, 1H; 3-H), 6.81 (dd, $J=10.7, 15.0$ Hz, 1H; 5-H), 6.29 (dd, $J=11.4, 15.0$ Hz, 1H; 4-H), 6.14 (d, $J=10.7$ Hz, 1H; 6-H), 5.86 (d, $J=15.3$ Hz, 1H; 2-H), 4.17 (q, $J=7.2$ Hz, 2H; CH_2CH_3), 2.28 (s, 3H; 8-H), 1.26 ppm (t, $J=7.2$ Hz, 3H; CH_3CH_2); ^{13}C NMR (125 MHz, CDCl_3): δ 166.9, 144.2, 136.2, 135.5, 130.5, 125.0, 121.7, 60.3, 26.9, 14.3 ppm; IR $\nu[\text{cm}^{-1}]$ =2982 (w), 2917 (w), 1693 (s), 1612 (s), 1580 (m), 1475 (w), 1443 (w), 1431 (w), 1366 (m), 1335 (m), 1298 (m), 1271 (m), 1233 (m), 1189 (m), 1141 (s), 1082 (s), 1013 (s), 963 (m), 894 (m), 870 (w), 836 (m), 720 (m), 647 (s), 580 (m); HRMS (ES^+) m/z [M+H] calcd for $\text{C}_{10}\text{H}_{14}\text{ClO}_2^+$ 201.06768, found 201.06709.

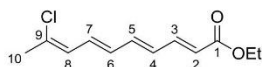
(2*E*,4*E*,6*Z*)-7-Chloro-octa-2,4,6-trien-1-ol **22**

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**22**

A stirred solution of ester **21** (1.157 g, 5.766 mmol, 1.00 eq) in dry CH_2Cl_2 (60 mL) at -78°C was treated with a 1 M DIBAL-H solution in *n*-hexane (12.7 mL, 12.685 mmol, 2.20 eq) and stirred for 1 h at this temperature. The mixture was allowed to warm to room temperature and aqueous citric acid (33% wt., 50 mL) was added. The phases were separated and the aqueous phase was extracted twice with CH_2Cl_2 (2×50 mL). The combined organic phases were dried (Na_2SO_4) and the solvent evaporated to give a colorless oil which was purified by column chromatography eluting with 25% EtOAc in *c*-hexane to furnish compound **22** (0.856 g, 5.396 mmol, 94%) as a colorless solid of m.p. 45.7°C ; R_f 0.16 (4:1 *c*-hexane / EtOAc); ^1H NMR (500 MHz, CDCl_3): δ 6.51 (dd, $J=10.5, 15.0$ Hz, 1H; 5-H), 6.34 (ddt, $J=1.3, 10.8, 15.0$ Hz, 1H; 4-H), 6.24 (dd, $J=10.8, 15.1$ Hz, 1H; 3-H), 6.10 (d, $J=10.5$ Hz, 1H; 6-H), 5.88 (dt, $J=5.9, 15.1$ Hz, 1H; 2-H), 4.21 (t, $J=5.5$ Hz, 2H; 1-H), 2.18 (s, 3H; 8-H), 1.36 ppm (t, $J=5.5$ Hz, 1H; OH); ^{13}C NMR (125 MHz, CDCl_3): δ 133.0, 132.5, 132.2, 131.6, 128.5, 125.4, 63.6, 26.7 ppm; IR $\nu[\text{cm}^{-1}]$ =3349 (br. m), 3025 (w), 2888 (w), 2840 (w), 1626 (m), 1587 (m), 1440 (m), 1378 (w), 1358 (m), 1315 (m), 1271 (m), 1235 (m), 1167 (w), 986 (s), 876 (m), 844 (m), 689 (m), 651 (s), 588 (m), 567 (m); HRMS (ES^+) m/z [M-OH] calcd for $\text{C}_8\text{H}_{10}\text{Cl}^+$ 141.04655, found 141.04619.

Ethyl (2E,4E,6E,8Z)-9-chloro-deca-2,4,6,8-tetraenoate 23

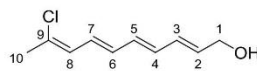
**23**

A stirred solution of alcohol **22** (0.856 g, 5.396 mmol, 1.00 eq) in dry CH_2Cl_2 (54 mL) at room temperature was treated with ylide **18** (2.256 g, 6.475 mmol, 1.20 eq) and MnO_2 (4.691 g, 53.962 mmol, 10.00 eq) and heated under reflux for 16 h. The mixture was allowed to cool to room temperature and the resulting suspension was filtered through a short plug of celite. The filtrate was concentrated to leave a red oil which was purified by column chromatography eluting with 5% EtOAc in *c*-hexane to afford **23** (0.729 g, 3.216 mmol, 60%) as a pale yellow solid of m.p. 93.0°C ; R_f 0.45 (4:1 *c*-hexane / EtOAc); ^1H NMR (300 MHz, CDCl_3): δ 7.31 (dd, $J=11.5, 15.3$ Hz, 1H; 3-H), 6.67 (dd, $J=10.6, 15.6$ Hz, 1H; 7-H), 6.61 (dd, $J=11.4, 14.8$ Hz, 1H; 5-H), 6.34 (dd, $J=11.5, 14.8$ Hz, 1H; 4-H), 6.30 (dd, $J=11.4, 15.6$ Hz, 1H; 6-H), 6.14 (d, $J=10.6$ Hz, 1H; 8-H), 5.87 (d, $J=15.3$ Hz, 1H; 2-H), 4.20 (q, $J=7.1$ Hz, 2H; CH_2CH_3), 2.20 (s, 3H; 10-H), 1.29 ppm (t, $J=7.1$ Hz, 3H; CH_3CH_2); ^{13}C NMR (75 MHz, CDCl_3): δ 167.1, 144.2, 140.5, 134.4, 132.3, 132.1, 130.7, 125.5, 121.3, 60.4, 26.9, 14.4 ppm; IR $\nu[\text{cm}^{-1}]$ =2980 (m), 2915 (w), 1706 (s), 1695 (s), 1618 (s), 1594 (m), 1443 (w), 1368 (m), 1350 (m), 1300 (m), 1252 (s), 1185

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(m), 1151 (m), 1133 (s), 1082 (w), 1035 (m), 1024 (m), 1010 (s), 976 (w), 851 (w), 720 (w), 655 (w); HRMS (ES⁺) m/z [M+H] calcd for C₁₂H₁₆ClO₂⁺ 227.08333, found 227.08293.

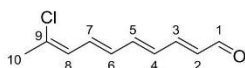
(2E,4E,6E,8Z)-9-Chloro-deca-2,4,6,8-tetraen-1-ol **24**



24

A stirred solution of ester **23** (0.089 g, 0.395 mmol, 1.00 eq) in dry CH₂Cl₂ (5 mL) at -78 °C was treated with a 1 M DIBAL-H solution in *n*-hexane (0.87 mL, 0.869 mmol, 2.20 eq) and stirred for 1 h at this temperature. The mixture was allowed to warm to room temperature and aqueous citric acid (33% wt., 10 mL) was added. The phases were separated and the aqueous phase was extracted twice with CH₂Cl₂ (2×20 mL). The combined organic phases were dried (Na₂SO₄) and the solvent was evaporated to give a colorless oil which was purified by column chromatography eluting with 20% EtOAc in *c*-hexane affording compound **24** (0.056 g, 0.303 mmol, 77%) as a white solid of m.p. 77.6 °C; R_f 0.18 (4:1 *c*-hexane / EtOAc); ¹H NMR (500 MHz, CDCl₃): δ 6.52 (dd, *J*=10.6, 14.8 Hz, 1H; 7-H), 6.35-6.23 (m, 4H; 3-H, 4-H, 5-H, 6-H), 6.11 (dd, *J*=0.9, 10.6 Hz, 1H; 8-H), 5.87 (dt, *J*=5.8, 14.9 Hz, 1H; 2-H), 4.22 (t, *J*=5.8 Hz, 2H; 1-H), 2.19 (s, 3H; 10-H), 1.35 ppm (t, br., *J*=5.6 Hz, 1H; OH); ¹³C NMR (125 MHz, CDCl₃): δ 133.4, 133.2, 132.8, 132.7, 132.1, 131.7, 128.7, 125.7, 63.6, 26.8 ppm; IR ν[cm⁻¹]=3259 (br. m), 3023 (w), 2892 (w), 2844 (w), 1624 (w), 1440 (m), 1430 (m), 1354 (m), 1282 (m), 1253 (w), 1226 (w), 1166 (w), 1131 (w), 1083 (m), 991 (s), 935 (m), 884 (m), 860 (m), 838 (m), 691 (m), 654 (m); HRMS (ES⁺) m/z [M-OH] calcd for C₁₀H₁₂Cl⁺ 167.06220, found 167.06171.

(2E,4E,6E,8Z)-9-Chloro-deca-2,4,6,8-tetraen-1-al **5**



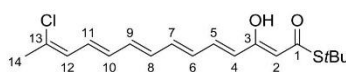
5

A stirred solution of alcohol **24** (0.056 g, 0.303 mmol, 1.00 eq) in dry CH₂Cl₂ (4 mL) at room temperature was treated with MnO₂ (0.527 g, 6.065 mmol, 20.00 eq) and stirred at this temperature for 16 h. The suspension was filtered through a short plug of celite and the filtrate was concentrated. The yellow oily remainder of crude **5** was immediately used for the next step without further purification. Only a small analytical sample was purified by column chromatography on silica gel eluting with 10% EtOAc in *c*-hexane and submitted to spectroscopy^[6] for a full characterization; R_f 0.39 (4:1 *c*-hexane / EtOAc); ¹H NMR (500 MHz, CDCl₃): δ 9.57 (d, *J*=7.9 Hz, 1H; 1-H), 7.13 (dd, *J*=11.2, 15.1 Hz, 1H; 3-H), 6.76 (dd, *J*=10.6,

S16

15.1 Hz, 1H; 7-H), 6.74 (dd, $J=11.2, 14.9$ Hz, 1H; 5-H), 6.47 (dd, $J=11.2, 14.9$ Hz, 1H; 4-H), 6.34 (dd, $J=11.2, 15.1$ Hz, 1H; 6-H), 6.20-6.12 (m, 2H; 2-H, 8-H), 2.22 ppm (s, 3H; 10-H); ^{13}C NMR (125 MHz, CDCl_3): δ 193.6, 151.6, 142.4, 135.6, 133.7, 132.0, 131.4, 130.6, 125.4, 27.1 ppm; IR $\nu[\text{cm}^{-1}]$ =3032 (w), 2916 (w), 2741 (w), 1667 (s), 1607 (w), 1584 (s), 1429 (w), 1379 (w), 1339 (w), 1282 (w), 1235 (w), 1158 (m), 1138 (m), 1110 (s), 1083 (m), 1009 (s), 990 (s), 891 (m), 841 (m), 806 (w), 748 (w), 656 (m); HRMS (ES^+) m/z $[\text{M}+\text{H}]$ calcd for $\text{C}_{10}\text{H}_{12}\text{ClO}^+$ 183.05712, found 183.05667.

S-*tert*-Butyl (2Z,4E,6E,8E,10E,12Z)-13-chloro-3-hydroxy-tetradeca-2,4,6,8,10,12-hexaenethioate 2



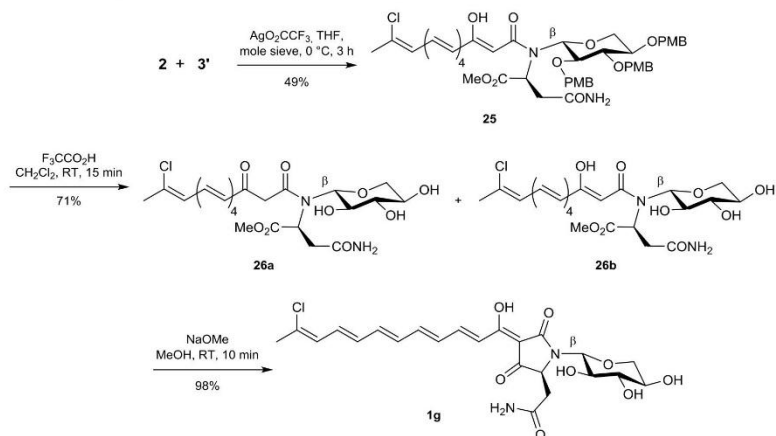
2

A stirred suspension of 60% NaH in mineral oil (0.040 g, 1.001 mmol, 3.30 eq) in dry THF (7 mL) at 0 °C was treated dropwise with phosphonate **4** (0.141 g, 0.455 mmol, 1.50 eq) and stirred at this temperature for an hour. Then the aldehyde **5** (0.055 g, 0.303 mmol, 1.00 eq), dissolved in dry THF (9 mL), was added to this mixture at 0 °C. The mixture was allowed to warm to room temperature over 16 h, saturated aqueous NH_4Cl solution was added, and the resulting mixture was extracted three times with diethyl ether (3×30 mL). The combined organic phases were dried (Na_2SO_4) and concentrated to give an orange solid which was purified by column chromatography eluting with 5% EtOAc in *c*-hexane to afford compound **2** (0.086 g, 0.254 mmol, 84% over 2 steps) as a reddish golden solid of m.p. 137.8 °C; R_f 0.72 (3:1 *c*-hexane / EtOAc); ^1H NMR (500 MHz, CDCl_3): δ 12.54 (d, $J=1.5$ Hz, 1H; OH), 7.15 (dd, $J=11.5, 15.0$ Hz, 1H; 5-H), 6.60 (dd, $J=10.6, 14.9$ Hz, 1H; 11-H), 6.54 (dd, $J=11.0, 14.8$ Hz, 1H; 9-H), 6.45 (dd, $J=11.2, 14.4$ Hz, 1H; 7-H), 6.36-6.26 (m, 3H; 6-H, 8-H, 10-H), 6.14 (d, $J=10.6$ Hz, 1H; 12-H), 5.79 (d, $J=15.0$ Hz, 1H; 4-H), 5.37 (s, 1H; 2-H), 2.20 (s, 3H; 14-H), 1.52 ppm (s, 9H; *t*Bu); ^{13}C NMR (125 MHz, CDCl_3): δ 196.1, 166.6, 138.9, 138.4, 136.4, 133.2, 133.1, 131.7, 130.2, 125.7, 125.1, 101.9, 48.6, 30.3, 26.9 ppm; IR $\nu[\text{cm}^{-1}]$ =2966 (w), 2912 (w), 2864 (w), 1630 (m), 1615 (m), 1563 (m), 1539 (m), 1477 (w), 1456 (m), 1411 (w), 1381 (m), 1361 (m), 1327 (m), 1295 (w), 1255 (m), 1202 (m), 1160 (m), 1132 (m), 1072 (s), 999 (s), 944 (m), 861 (s), 814 (m), 762 (s), 680 (m); HRMS (ES^+) m/z $[\text{M}+\text{H}]$ calcd for $\text{C}_{18}\text{H}_{24}\text{ClO}_2\text{S}^+$ 339.11800, found 339.11768.

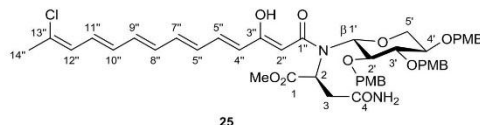
S17

2.4. Synthesis of aurantoside G (1g)

Overview of the synthesis of aurantoside G (1g)



Methyl (2S)-(2''Z,4''E,6''E,8''E,10''E,12''Z)-N-(2',3',4'-tri-O-(4-methoxybenzyl)-β-D-xylopyranosyl)-N-(13"-chloro-1"-oxo-3"-hydroxy-tetradeca-2",4",6",8",10",12"-hexaenyl)-2-amino-4-oxo-4-aminobutanoate 25

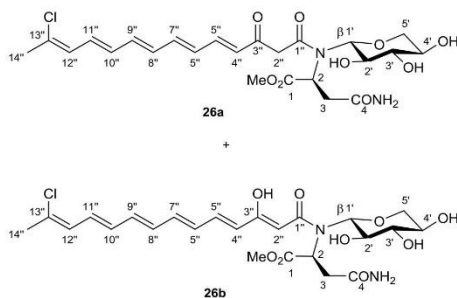


A mixture of *N*-D-asparaginate **3'** (0.133 g, 0.208 mmol, 1.00 eq), thioate **2** (0.088 g, 0.260 mmol, 1.25 eq), freshly dried, powdered mole sieve (0.300 g), and dry THF (4.6 mL) at 0°C was slowly treated with a solution of silver trifluoroacetate (0.073 g, 0.332 mmol, 1.60 eq) in dry THF (1 mL) and stirred for 3 h at this temperature. The mixture was diluted with diethyl ether to precipitate most of the silver salt, and filtered through a short plug of celite. A saturated aqueous solution of NH_4Cl was added to the filtrate, the organic phase was separated and the aqueous phase was extracted three times with diethyl ether (3×15 mL). The combined organic phases were dried (Na_2SO_4) and concentrated in vacuo to give a yellow oil which was purified by column chromatography eluting with EtOAc 50% in *c*-hexane affording compound **25** (0.036 g, 0.0406 mmol, 49% based on recovered *N*-D-asparaginate **3'**) as an orange amorphous solid; $[\alpha]_D^{25} = 58.8$ ($c = 1.00$, CHCl_3); R_f 0.49 (1:3 *c*-hexane / EtOAc); ^1H NMR (300 MHz, CDCl_3): ill resolved and not assigned; ^{13}C NMR (75 MHz, CDCl_3): not assigned; IR $\nu[\text{cm}^{-1}] = 2915$ (w), 1737 (w), 1683 (m), 1614 (m), 1583 (m), 1514 (s), 1464 (m), 1345 (m), 1303 (m), 1268 (s),

S18

1174 (m), 1081 (s), 1034 (m), 1005 (m), 950 (w), 831 (m); HRMS (ES⁺) *m/z* [M+H] calcd for C₄₈H₅₆N₂ClO₁₂⁺ 887.35163, found 887.35065.

Methyl (2*S*)-(2''*Z*,4''*E*,6''*E*,8''*E*,10''*E*,12''*Z*)-(N-β-D-xylopyranosyl)-N-(13''-chloro-1''-oxo-3''-hydroxy-tetradeca-2'',4'',6'',8'',10'',12''-hexaenyl)-2-amino-4-oxo-4-aminobutanoate
26

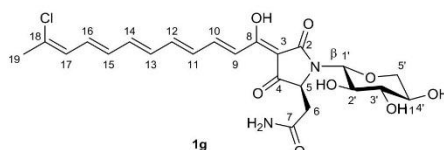


A stirred solution of protected β-ketoamide **25** (0.046 g, 0.052 mmol, 1.00 eq) in CH₂Cl₂ (2.4 mL) at 0 °C was treated with anisole (0.034 mL, 0.314 mmol, 6.00 eq) and dropwise with F₃CCO₂H (0.24 mL). The mixture was stirred for another 15 min at this temperature and then diluted with toluene. Acid was removed azeotropically by repeated co-evaporation with toluene (5×15 mL). The residual yellow oil was purified by column chromatography eluting with 10% methanol in dichloromethane and spectra of the eluates were recorded immediately.^[6] The keto and enol tautomers could be separated in a pure state; **26b** (enol): 10.3 mg (0.0195 mmol, 38%); *R_f* 0.35 (9:1 CH₂Cl₂ / MeOH); [α]_D²⁵ = -21.0 (c=0.10, MeOH); ¹H NMR (500 MHz, MeOD): δ 7.26 (dd, *J*=11.5, 14.9 Hz, 1H; 5''-H), 6.61 (dd, *J*=10.9, 15.2 Hz, 1H; 7''-H), 6.09 (d, *J*=14.9 Hz, 1H; 4''-H), 6.59 (dd, *J*=10.5, 16.1 Hz, 1H; 11''-H), 6.36-6.52 (m, 4H; 6''-H, 8''-H, 9''-H, 10''-H), 6.26 (d, *J*=10.5 Hz, 1H; 12''-H), 5.22 (s, 1H; 2''-H), 4.83 (d, *J*=8.4 Hz, 1H; 1'-H), 4.75 (d, br., *J*=6.5 Hz, 1H; 2-H), 3.95 (dd, *J*=5.6, 11.5 Hz, 1H; 5'-H^a), 3.84 (dd, *J*=8.4, 8.7 Hz, 1H; 2'-H), 3.75 (dd, *J*=8.7, 8.9 Hz, 1H; 3'-H), 3.64 (s, 3H; OMe), 3.63-3.56 (m, 1H; 4'-H), 3.37 (dd, *J*=6.3, 8.9 Hz, 1H; 5'-H^b), 3.36 (d, *J*=10.9 Hz, 1H; 3-H^a), 2.50 (dd, *J*=3.7, 15.9 Hz, 1H; 3-H^b), 2.19 ppm (s, 3H; 14''-H); ¹³C NMR (125 MHz, MeOD): δ 176.1, 172.5, 166.3, 164.7, 139.6, 138.9, 137.1, 134.8, 134.7, 133.5, 133.2, 130.7, 126.9, 126.3, 104.0, 89.2, 84.8, 76.7, 71.1, 68.4, 58.2, 52.7, 38.4, 26.7 ppm; IR ν[cm⁻¹]=3340 (br. w), 2915 (w), 1736 (m), 1665 (m), 1620 (s), 1597 (s), 1567 (m), 1439 (m), 1349 (m), 1326 (m), 1205 (w), 1081 (m), 1054 (m), 1006 (s), 825 (w); HRMS (ES⁺) *m/z* [M-OH] calcd for C₂₄H₃₀N₂ClO₈⁺ 509.16852, found 509.16833; **26a** (keto): 9.3 mg (0.0176 mmol, 34%); *R_f* 0.16 (9:1 CH₂Cl₂ / MeOH); [α]_D²⁵ = -24.7 (c=0.10, MeOH); ¹H NMR (500 MHz, MeOD): δ 7.38 (dd, *J*=11.2, 15.5 Hz, 1H; 5''-H), 7.08 (dd, *J*=11.4, 15.1 Hz, 1H; 7''-H), 6.84 (dd, *J*=11.1, 14.7 Hz, 1H; 11''-H), 6.50-6.35 (m, 3H; 8''-H, 9''-H, 10''-H), 6.27 (dd,

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$J=11.4$, 11.8 Hz, 2H; $12''$ -H, $6''$ -H), 6.22 (d, $J=15.4$ Hz, 1H; $4''$ -H), 4.94 - 4.86 (m, 1H; $1'$ -H), 4.69 (dd, $J=2.5$, 9.6 Hz, 1H; 2-H), 3.94 (dd, $J=4.8$, 5.3 Hz, 1H; $5'$ -H^a), 3.93 (dd, $J=4.7$, 5.3 Hz, 1H; $2''$ -H^a), 3.63 (s, 3H; OMe), 3.58 - 3.52 (m, 1H; $4'$ -H), 3.52 - 3.32 (m, 4H; 3-H^a, $2'$ -H, $3'$ -H, $5'$ -H^b), 3.28 - 3.18 (m, 1H; $2''$ -H^b), 2.55 (dd, $J=2.7$, 16.1 Hz, 1H; 3-H^b), 2.20 ppm (s, 3H; $14''$ -H); ^{13}C NMR (125 MHz, MeOD): δ 195.4, 175.8, 172.6, 170.5, 146.7, 144.2, 139.4, 134.5, 134.4, 134.1, 132.0, 129.6, 126.9, 126.8, 89.0, 79.1, 72.4, 70.7, 69.3, 69.3, 53.2, 52.9, 37.3, 26.7 ppm; HRMS (ES⁺) m/z [M+H] calcd for $\text{C}_{24}\text{H}_{32}\text{N}_2\text{ClO}_9$ ⁺ 527.17908, found 527.17898.

Aurantioside G (1g)



A stirred solution of unprotected β -ketoamide **26a** (9.3 mg, 0.0176 mmol, 1.00 eq) in MeOH (2 mL) at room temperature was treated with NaOMe (3.81 mg, 0.0706 mmol, 4.00 eq) and stirred for a further 10 min at this temperature. The mixture was acidified with 1 M HCl (pH~2), the phases were separated, and the aqueous one was extracted ten times with CH_2Cl_2 until its color had vanished. The combined organic phases were dried (Na_2SO_4) and concentrated to afford aurantioside G (**1g**) (8.54 mg, 0.0172 mmol, 98%) as a deep-red amorphous solid, prone to decomposition. All spectra and analytical data were recorded as fast as possible; $[\alpha]_{\text{D}}^{25} = -132.0$ ($c=0.10$, MeOH) (loc. cit.^[8]: -116); ^1H NMR (300 MHz, MeOD): δ 7.63 (dd, $J=11.7$, 14.1 Hz, 1H; 10-H), 7.23 (d, $J=14.9$ Hz, 1H; 9-H), 6.89 (dd, $J=11.5$, 13.1 Hz, 1H; 12-H), 6.73-6.38 (m, 5H; 11-H, 13-H, 14-H, 15-H, 16-H), 6.30 (d, $J=10.5$ Hz, 1H; 17-H), 4.82 (d, $J=9.2$ Hz, 1H; $1'$ -H), 4.31 (s, br., 1H; 5-H), 4.02 (dd, $J=8.9$ Hz, 1H; $2'$ -H), 3.89 (dd, $J=5.3$, 11.1 Hz, 1H; $5'$ -H^a), 3.35-3.31 (m, 2H; $3'$ -H, $4'$ -H), 3.26 (dd, $J=11.1$ Hz, 1H; $5'$ -H^b), 2.83 (s, br., 2H; 6-H), 2.21 ppm (s, 3H; 19-H); ^{13}C NMR (75 MHz, MeOD): δ 175.7, 174.3, 146.8, 145.5, 140.4, 134.8, 134.6, 134.3, 132.6, 132.5, 126.8, 121.8, 101.9, 85.5, 79.2, 71.4, 70.9, 69.4, 62.5, 37.9, 26.7 ppm; IR $\nu[\text{cm}^{-1}]$ =3337 (br. w), 1608 (m), 1559 (m), 1420 (br. m), 1051 (s), 1006 (s); HRMS (ES⁺) m/z [M+H] calcd for $\text{C}_{23}\text{H}_{28}\text{N}_2\text{ClO}_8$ ⁺ 495.15287, found 495.15146; UV (MeCN) λ_{max} 454 nm, 305 nm, 276 nm. All data, save for the UV absorption band, is in accordance with the literature, which shows compared to aurantioside H (**1h**) and aurantioside I (**1i**) inconsistent values for the maximum absorption of about 430 nm and consequently must be a typo.^[8]

2.5. HPLC chromatogram and UV spectrum of synthetic 1g

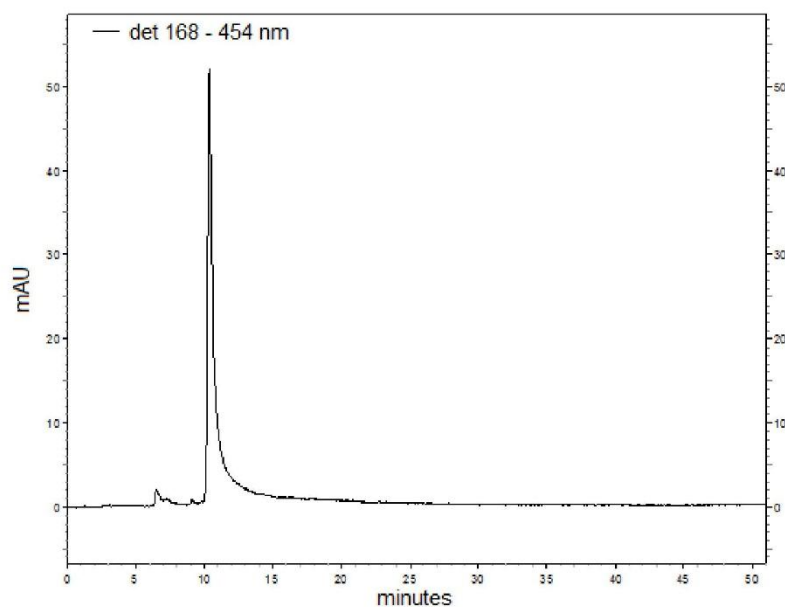


Figure 1. HPLC chromatogram of auranoside G (**1g**) (Phenomenex Kinetex 250x4.6 mm, mobile phase 40% MeCN, 60% H₂O with 0.1% HCOOH for 10 min to 100 % MeCN in 25 min, flow rate 0.7 mL/min).

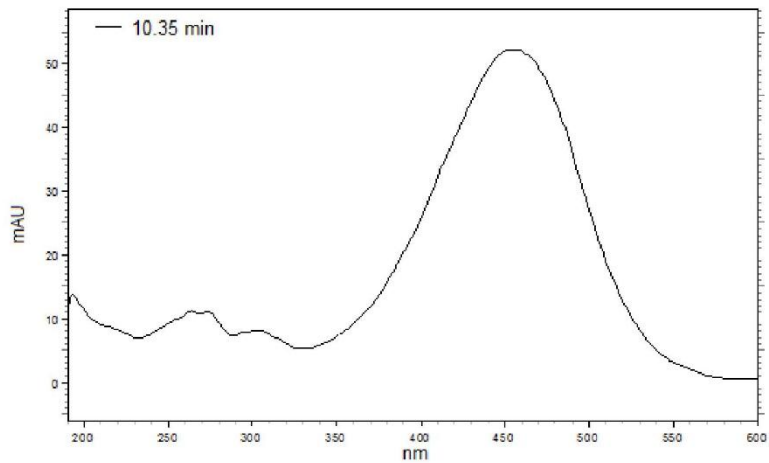


Figure 2. Absorption maxima of auranoside G (**1g**).

Comparison of spectra of the natural isolate of **1g** (Ireland) and synthetic **1g** (Schobert)

| atom | ¹ H signals | | ¹³ C signals | |
|------|--|---|--------------------------|---------------------------|
| | Ireland group δ (ppm), multipl., J (Hz) | Schobert group δ (ppm), multipl., J (Hz) | Ireland group δ (ppm) | Schobert group δ (ppm) |
| 1 | | | | |
| 2 | | | 174.9 | Not observed |
| 3 | | | Not observed | 101.9 |
| 4 | | | 201.7 | Not observed |
| 5 | 4.30, br s | 4.31, br s | 63.0 | 62.5 |
| 6a | 2.80, br m | 2.83, br s | 38.0 | 37.9 |
| 6b | 2.80, br m | 2.83, br s | | |
| 7 | | | 174.2 | 174.3 |
| 8 | | | 175.2 | 175.7 |
| 9 | 7.23, br d, 15.3 | 7.23, d, 14.9 | 121.5 | 121.8 |
| 10 | 7.63, dd, 15.3/11.2 | 7.63, dd, 14.1/11.7 | Not assigned | 146.8 |
| 11 | 6.55, dd, 14.3/11.1 | 6.38-6.73, m | 132.6 | 132.6 |
| 12 | 6.87, dd, 14.3/11.0 | 6.89, dd, 13.1/11.5 | 145.5 | 145.5 |
| 13 | 6.48, m | 6.38-6.73, m | 134.7 | 134.8 |
| 14 | 6.66, m | 6.38-6.73, m | 140.5 | 140.4 |
| 15 | 6.49, m | 6.38-6.73, m | 134.3 | 134.3 |
| 16 | 6.68, dd, 14.5, 10.4 | 6.38-6.73, m | 132.5 | 132.5 |
| 17 | 6.31, d, 10.4 | 6.30, d, 10.5 | 126.8 | 126.8 |
| 18 | | | 134.5 | 134.6 |
| 19 | 2.21, s | 2.21, s | 26.8 | 26.7 |
| 1' | 4.77 d, 8.8 | 4.82, d, 9.2 | 85.6 | 85.5 |
| 2' | 4.03, dd, 8.8/8.8 | 4.02, dd, 8.9/8.9 | 71.4 | 71.4 |
| 3' | 3.32, br dd, 8.8/8.8 | 3.31-3.35, m | 79.2 | 79.2 |
| 4' | 3.55, br dd, 10.8/8.8/5.3 | 3.31-3.35, m | 70.9 | 70.9 |
| 5'a | 3.24, dd, 11.1/10.8 | 3.26, dd, 11.1/11.0 | 69.4 | 69.4 |
| 5'b | 3.88, dd, 11.1/5.3 | 3.89, dd, 11.1/5.3 | | |

3. References

- [1] H. E. Gottlieb, V. Kotlyar, A. Nudelman, *J. Org. Chem.* **1997**, *62*, 7512-7515.
- [2] Z. Huang, M. Zhang, S. D. Burton, L. N. Katsakhyan, H. Ji, *ACS Chem. Biol.* **2014**, *9*, 193-201.
- [3] E. Biron, H. Kessler, *J. Org. Chem.* **2005**, *70*, 5183-5189.
- [4] B. K. Gorityala, J. Ma, K. K. Pasunooti, S. Cai, X.-W. Liu, *Green Chem.* **2011**, *13*, 573-577.
- [5] S. V. Ley, P. R. Woodward, *Tetrahedron Lett.* **1987**, *28*, 345-346.
- [6] O. Hartmann, M. Kalesse, *Angew. Chem. Int. Ed.* **2014**, *53*, 7335-7338.
- [7] N. A. Heaps, C. D. Poulter, *J. Org. Chem.* **2011**, *76*, 1838-1843.
- [8] A. S. Ratnayake, R. A. Davis, M. K. Harper, C. A. Veltri, C. D. Andjelic, L. R. Barrows, C. M. Ireland, *J. Nat. Prod.* **2005**, *68*, 104-107.

4.1. Intermediates enroute to amino acid 7

CCOC(=O)C(=O)C(C)C(=O)Nc1ccc(C)cc1
 10

file: ...lonNMRJahr_1MP252_Fr3-201010fid exp: <zg30>
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S24

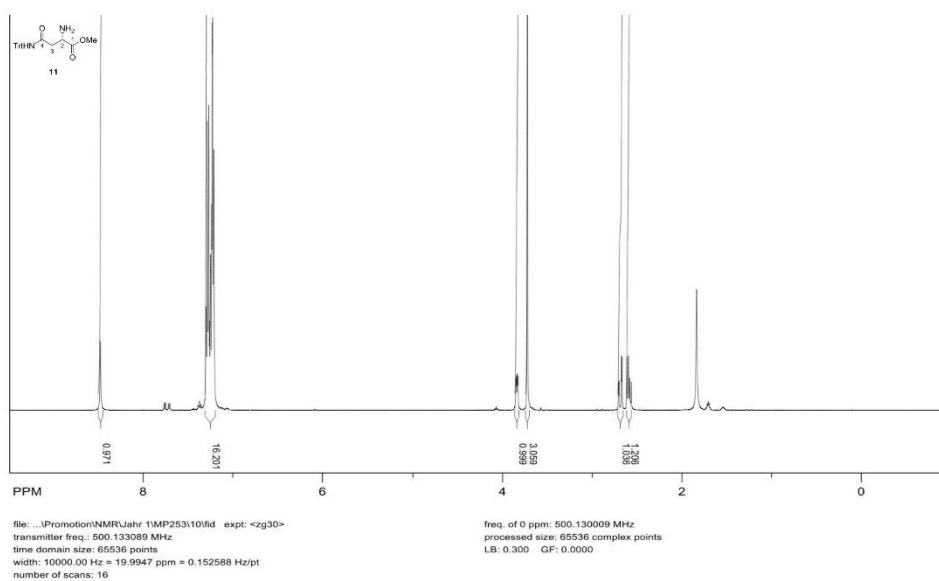
Chemical structure of compound 10 is shown above the spectrum. The structure is a substituted benzamide derivative with a trifluoromethyl group and a methoxycarbonyl group.

The spectrum displays the following chemical shifts (ppm) for the peaks:

- 171.619
- 169.448
- 156.360
- 143.870
- 143.868
- 143.866
- 143.864
- 143.862
- 143.860
- 143.858
- 143.856
- 143.854
- 143.852
- 143.850
- 143.848
- 143.846
- 143.844
- 143.842
- 143.840
- 143.838
- 143.836
- 143.834
- 143.832
- 143.830
- 143.828
- 143.826
- 143.824
- 143.822
- 143.820
- 143.818
- 143.816
- 143.814
- 143.812
- 143.810
- 143.808
- 143.806
- 143.804
- 143.802
- 143.800
- 143.798
- 143.796
- 143.794
- 143.792
- 143.790
- 143.788
- 143.786
- 143.784
- 143.782
- 143.780
- 143.778
- 143.776
- 143.774
- 143.772
- 143.770
- 143.768
- 143.766
- 143.764
- 143.762
- 143.760
- 143.758
- 143.756
- 143.754
- 143.752
- 143.750
- 143.748
- 143.746
- 143.744
- 143.742
- 143.740
- 143.738
- 143.736
- 143.734
- 143.732
- 143.730
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- 143.726
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- 143.416
- 143.414
- 143.412
- 143.410
- 143.408
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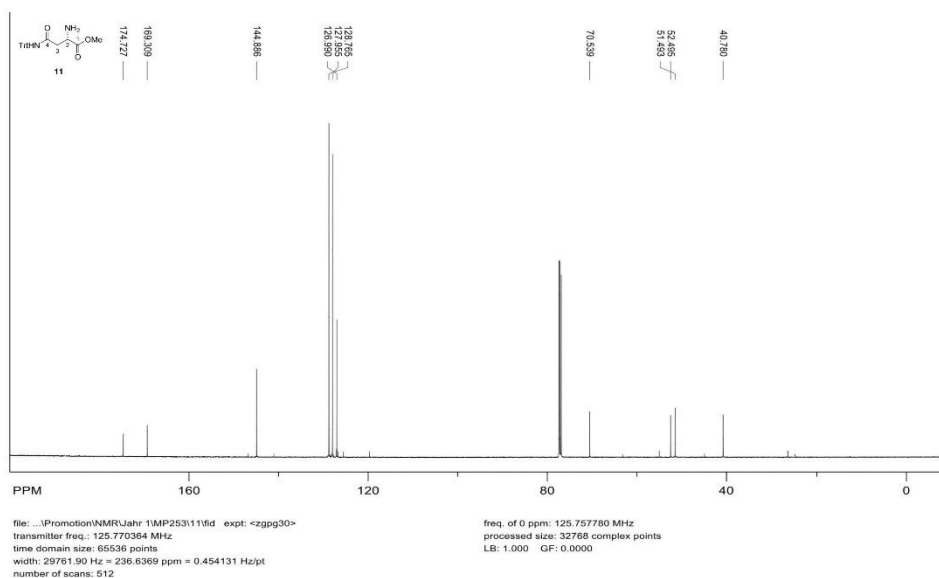
S25

¹H NMR spectrum for methyl (S)-2-amino-4-oxo-4-(tritylamino)butanoate 11 (500 MHz, CDCl₃)



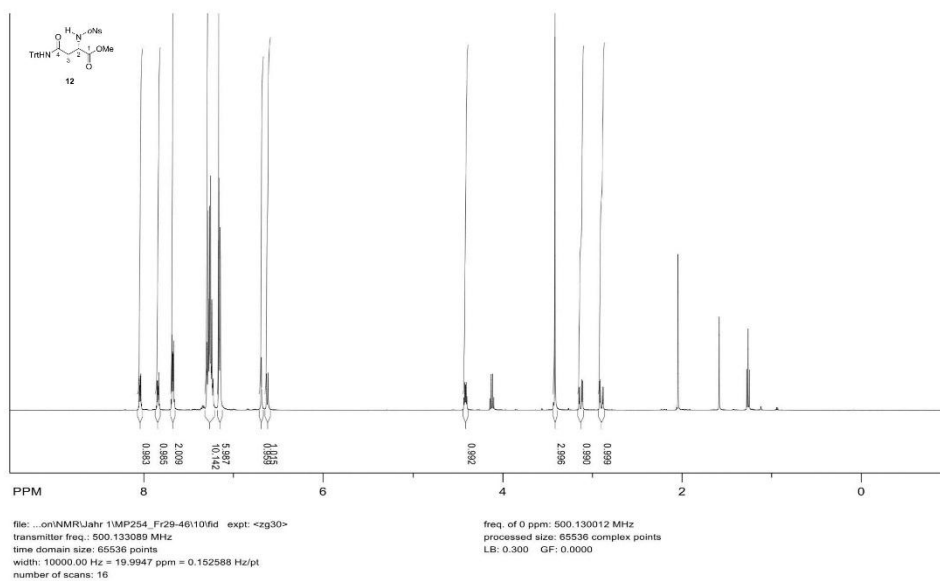
S26

¹³C NMR spectrum for methyl (S)-2-amino-4-oxo-4-(tritylamino)butanoate 11 (125 MHz, CDCl₃)



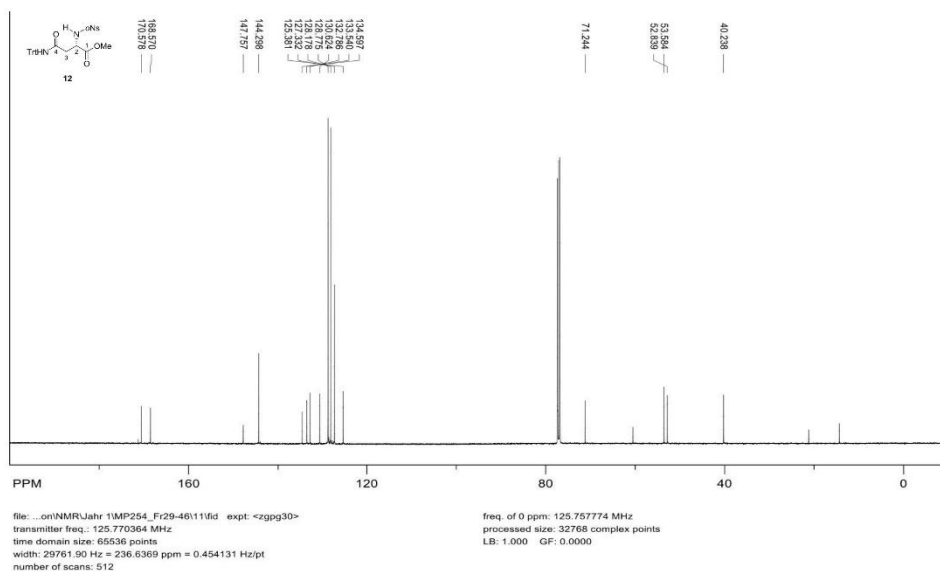
S27

¹H NMR spectrum for methyl (S)-2-(o-nitrobenzenesulfonylamino)-4-oxo-4-(tritylamino)butanoate 12 (500 MHz, CDCl₃)



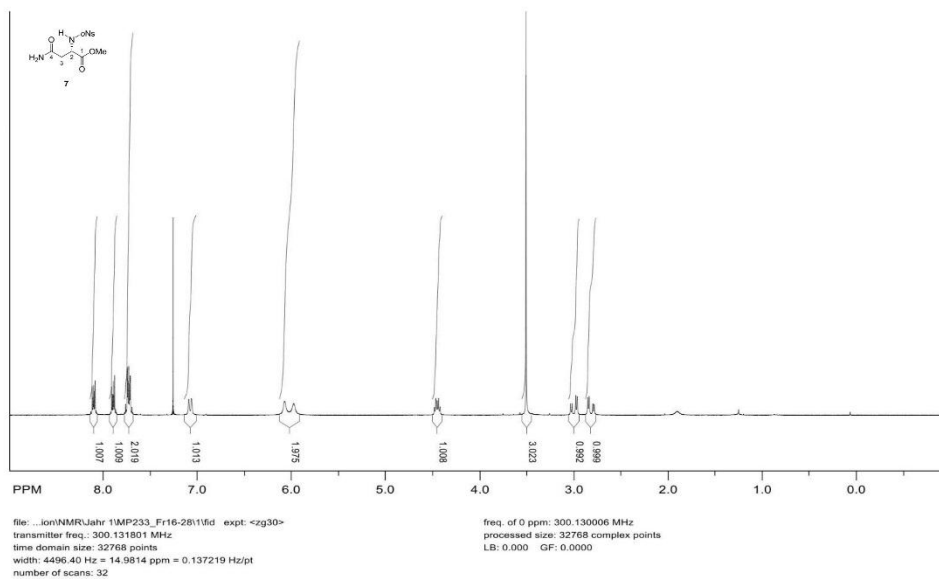
S28

¹³C NMR spectrum for methyl (S)-2-(o-nitrobenzenesulfonylamino)-4-oxo-4-(tritylamino)butanoate 12 (125 MHz, CDCl₃)



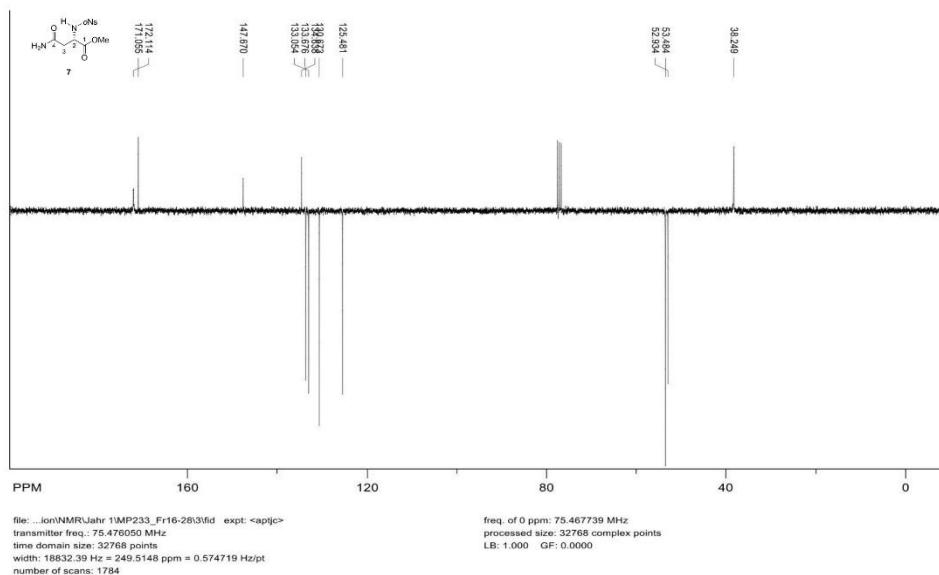
S29

¹H NMR spectrum for methyl (S)-2-(o-nitrobenzenesulfonylamino)-4-oxo-4-aminobutanoate 7 (300 MHz, CDCl₃)



S30

¹³C NMR spectrum for methyl (S)-2-(o-nitrobenzenesulfonylamino)-4-oxo-4-aminobutanoate 7 (75 MHz, CDCl₃)



S31

¹H NMR spectrum for 1-O-allyl-D-xylopyranoside 13 (300 MHz, CDCl₃)



CC(C=C)OC(=O)C1C(C(C(C1)OC)OC)OC

14

file: ...4-Tri-O-PMB-1-O-allylxytose 11f1d exp: <zg30>
 transmitter freq.: 300.131801 MHz
 time domain size: 32768 points
 width: 4496.40 Hz = 14.9814 ppm = 0.137219 Hz/pt
 number of scans: 32

freq. of 0 ppm: 300.130011 MHz
 processed size: 32768 complex points
 LB: 0.000 GF: 0.0000

S34

Chemical Structure:

COC1=CC=C(C(=C1)OCCOC/C=C/C)OCCOC/C=C/C

Peak List (ppm):

- 77.830
- 77.729
- 77.516
- 77.316
- 77.164
- 77.055
- 69.448
- 69.386
- 60.144
- 55.280
- 159.448
- 159.254
- 159.254
- 159.250
- 133.808
- 133.781
- 133.716
- 133.688
- 113.888
- 103.564
- 95.397

File Information:

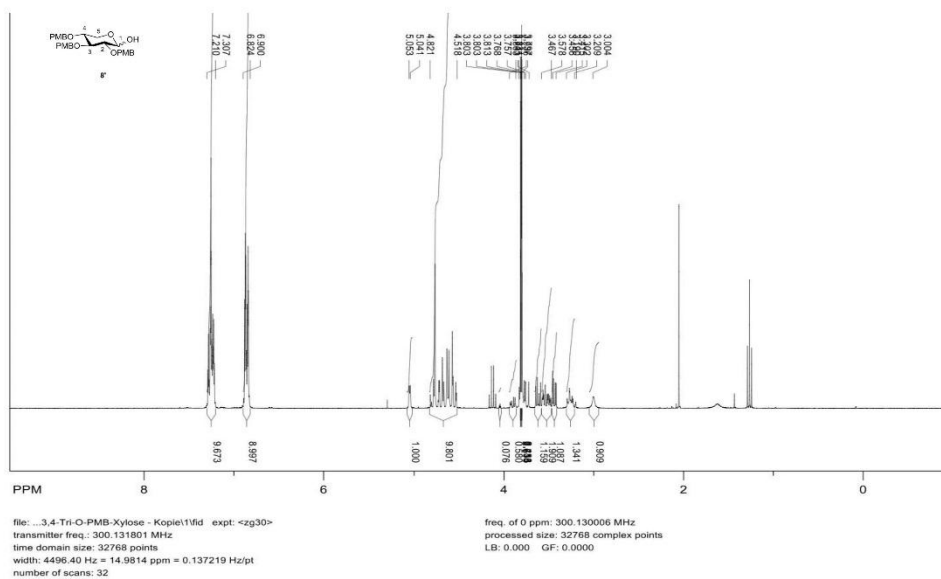
```
file: ...;4-Tri-O-PMB-1-O-allylxlyose\3\fid exp: <aplj>
transmitter freq.: 75.476050 MHz
time domain size: 32768 points
width: 18832.99 Hz = 249.5148 ppm = 0.574719 Hz/pt
number of scans: 540
```

Acquisition Parameters:

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freq. of 0 ppm: 75.467749 MHz
processed size: 32768 complex points
LB: 1.000 GF: 0.0000
```

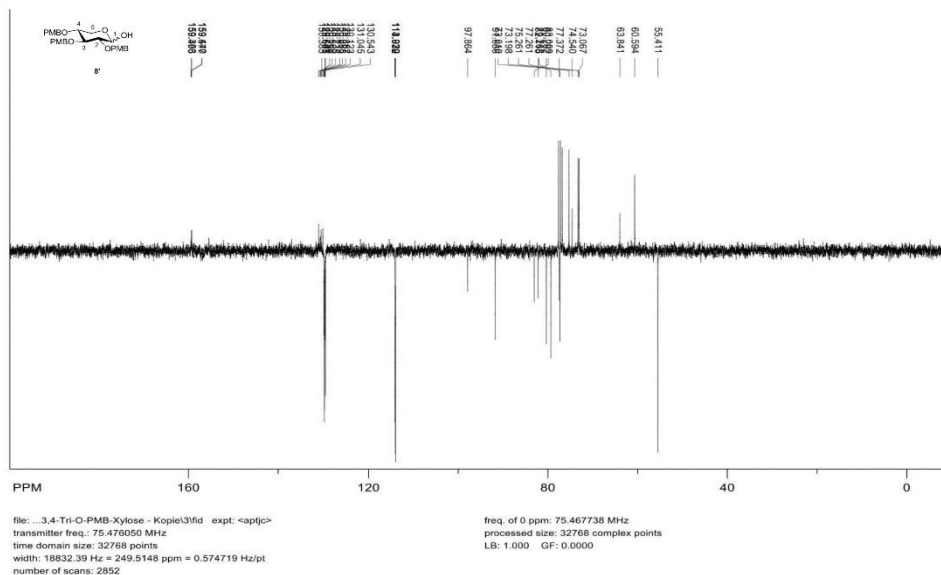
S35

¹H NMR spectrum for 2,3,4-tri-*O*-(4-methoxybenzyl)-*D*-xylopyranoside 8' (300 MHz, CDCl₃)



S36

¹³C NMR spectrum for 2,3,4-tri-*O*-(4-methoxybenzyl)-D-xylopyranoside 8' (75 MHz, CDCl₃)

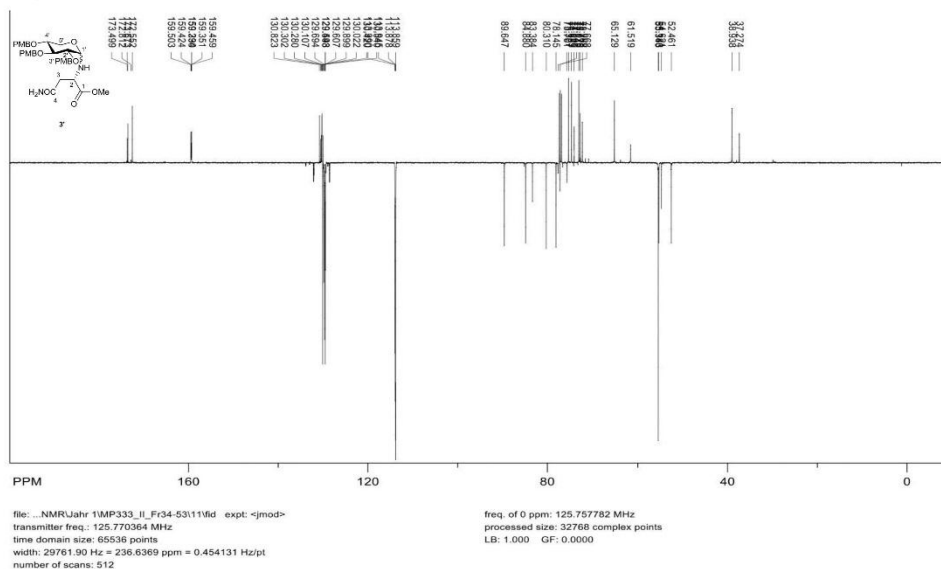


S37

file: ...NMRJahr 1MP333_II_Fr34-53;10.fid expf: <zg30>
transmitter freq.: 500.133089 MHz
time domain size: 65536 points
width: 10000.00 Hz = 19.9947 ppm = 0.152588 Hz/pt
number of scans: 64

freq. of 0 ppm: 500.130011 MHz
processed size: 65536 complex points
LB: 0.300 GF: 0.0000

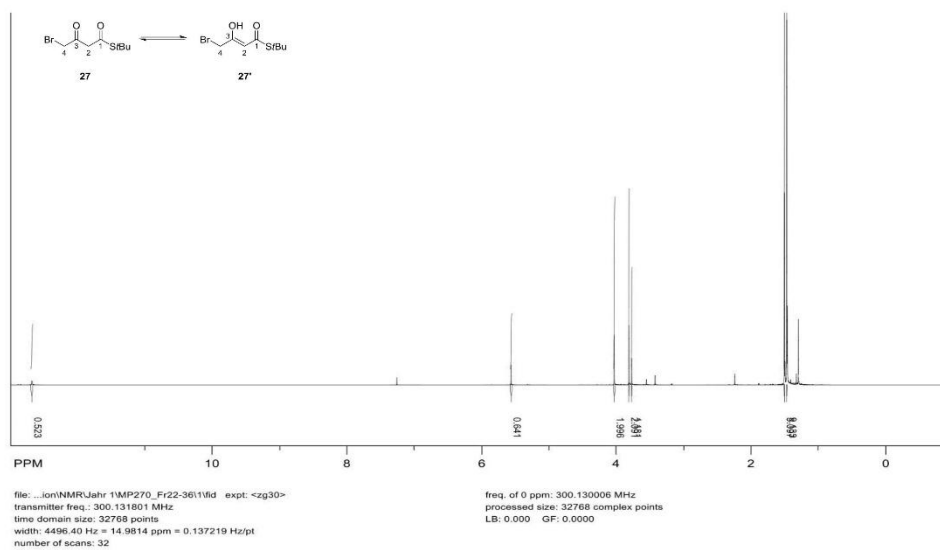
¹³C NMR spectrum for methyl (2S)-N-(2',3',4'-tri-O-(4-methoxy-benzyl)-D-xylopyranosyl)-amino-4-oxo-4-aminobutanoate 3' (125 MHz, CDCl₃)



S41

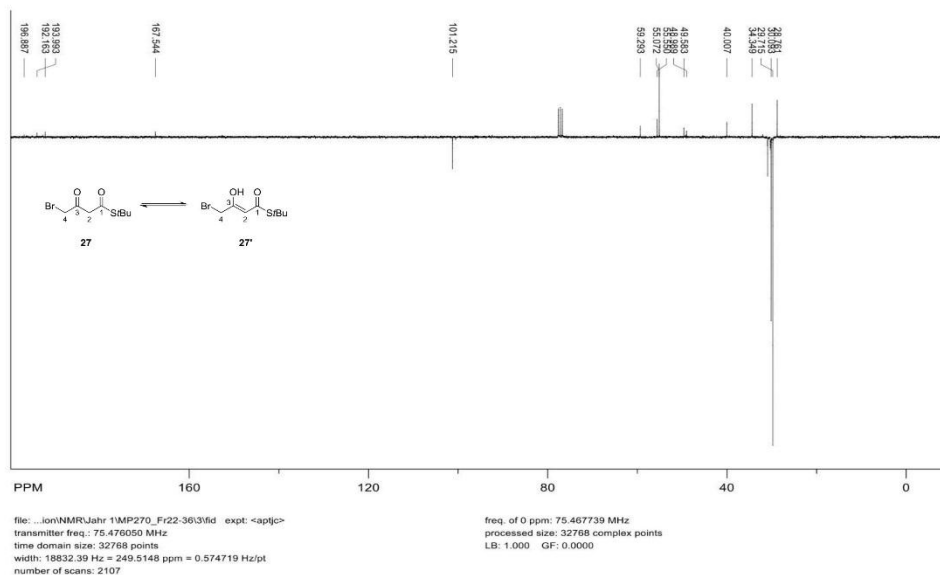
4.3. Intermediates enroute to sidechain 2

¹H NMR spectrum for *S*-tert-butyl-4-bromo-3-oxobutanethioate 27 (300 MHz, CDCl₃)



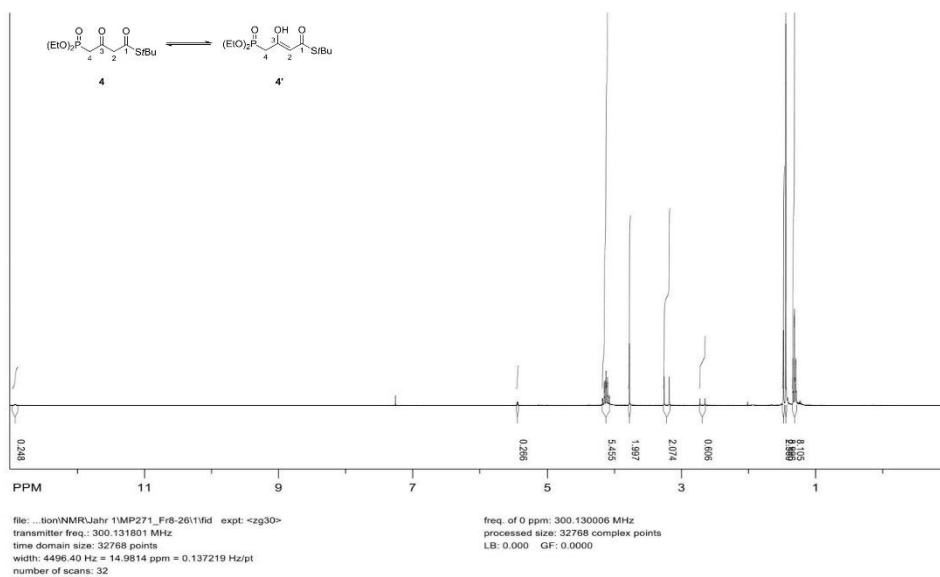
S42

¹³C NMR spectrum for *S*-tert-butyl-4-bromo-3-oxobutanethioate 27 (75 MHz, CDCl₃)



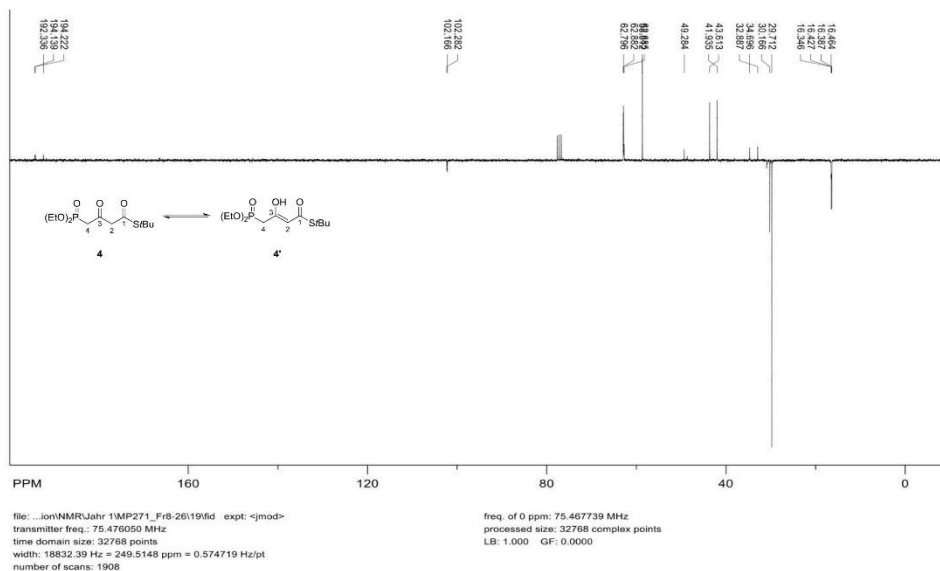
S43

¹H NMR spectrum for S-tert-butyl-4-(diethoxyphosphono)-3-oxobutanethioate 4 (300 MHz, CDCl₃)



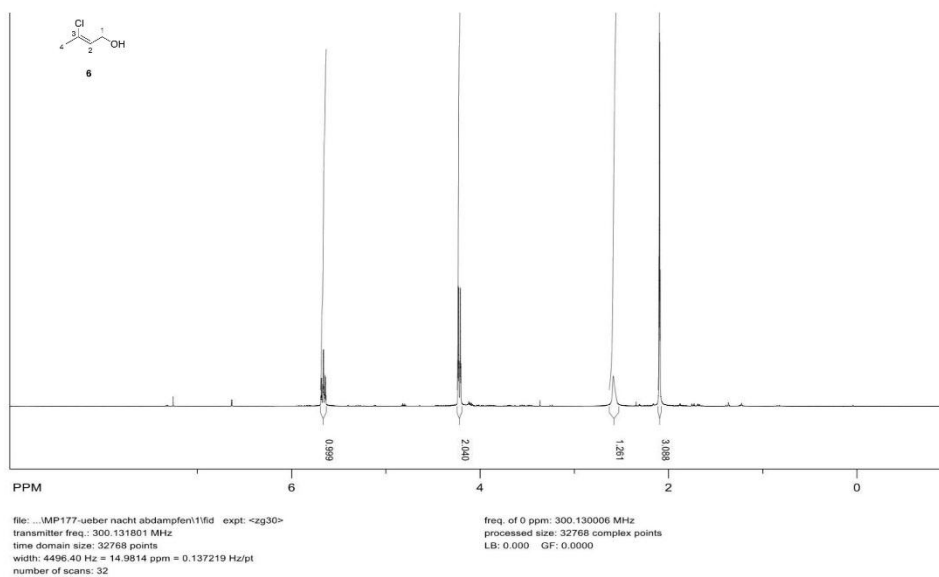
S44

¹³C NMR spectrum for S-tert-butyl-4-(diethoxyphosphono)-3-oxobutanethioate 4 (75 MHz, CDCl₃)



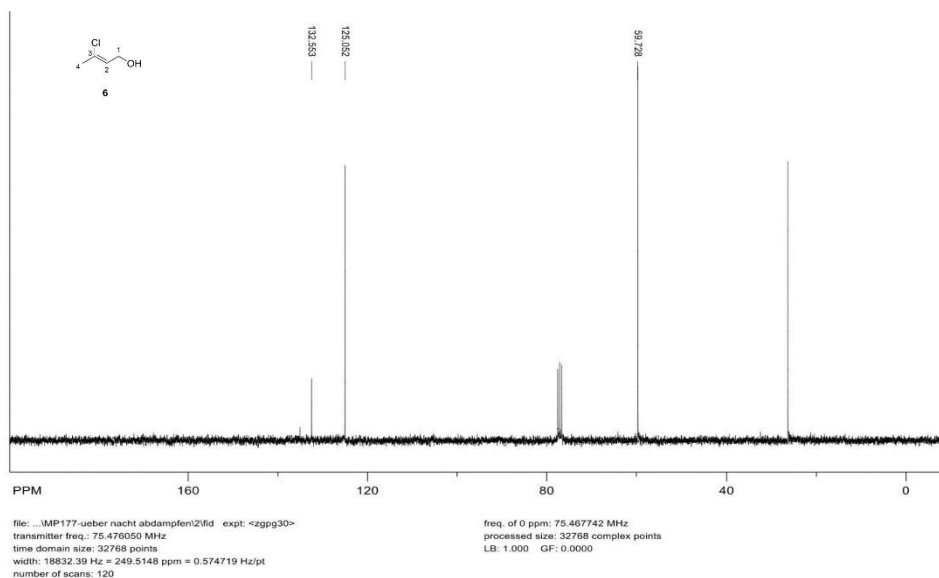
S45

¹H NMR spectrum for (Z)-3-chloro-but-2-en-1-ol **6** (300 MHz, CDCl₃)



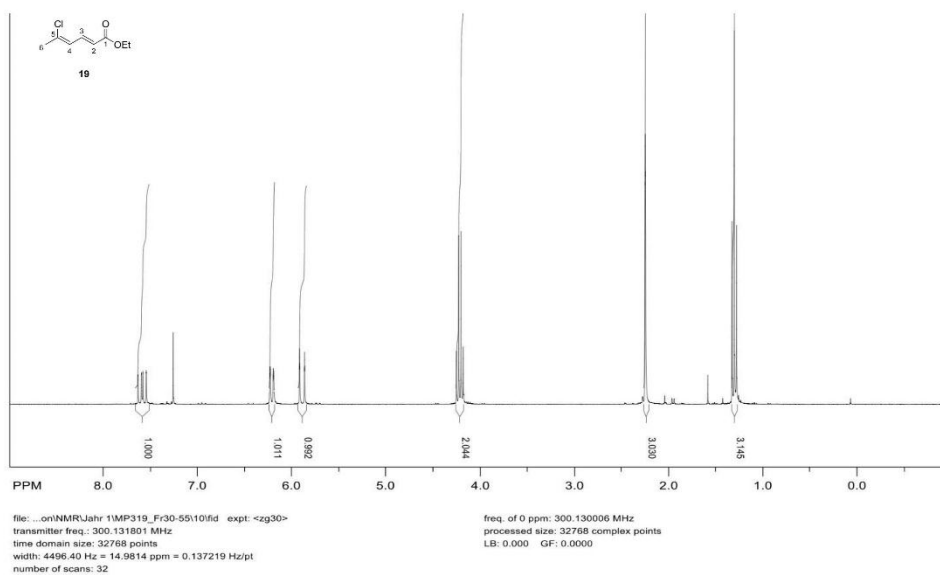
S46

¹³C NMR spectrum for (Z)-3-chloro-but-2-en-1-ol **6** (75 MHz, CDCl₃)



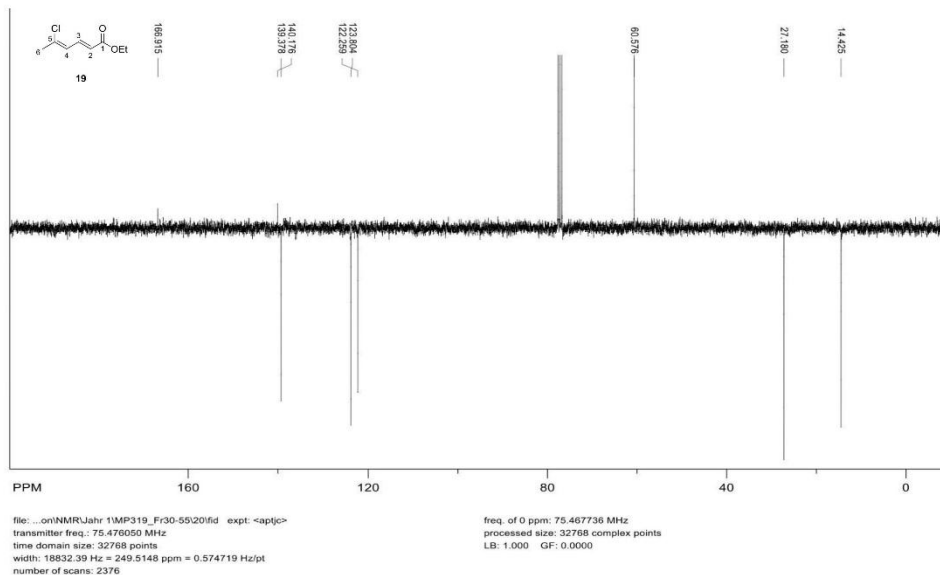
S47

¹H NMR spectrum for (2E,4Z) ethyl-5-chloro-hexa-2,4-dienoate 19 (300 MHz, CDCl₃)



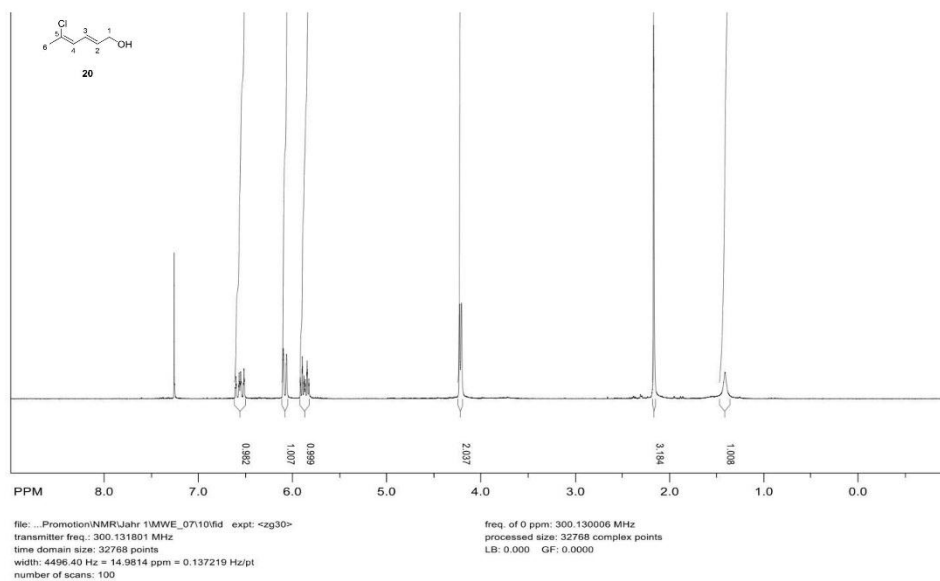
S48

¹³C NMR spectrum for (2E,4Z) ethyl-5-chloro-hexa-2,4-dienoate 19 (75 MHz, CDCl₃)



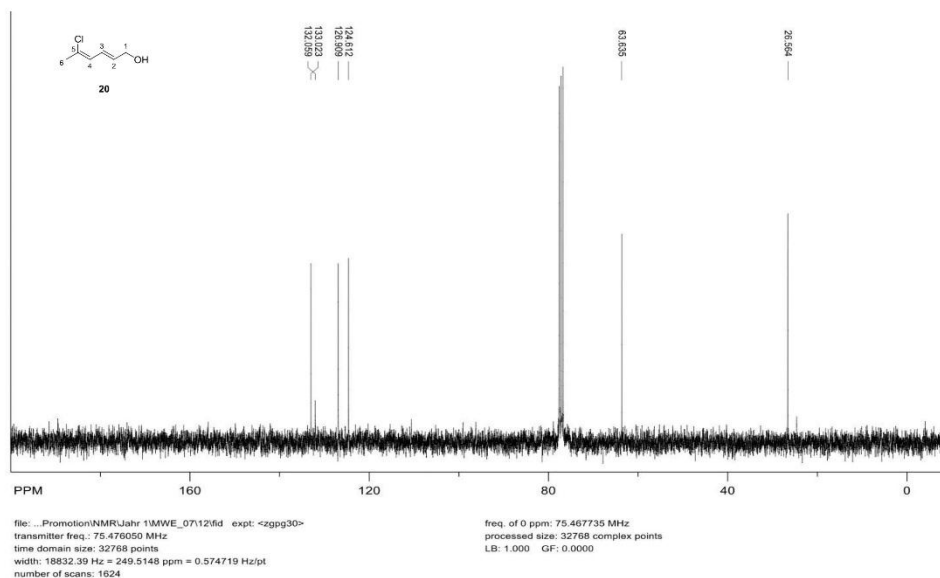
S49

¹H NMR spectrum for (2E,4Z) 5-chloro-hexa-2,4-dien-1-ol 20 (300 MHz, CDCl₃)



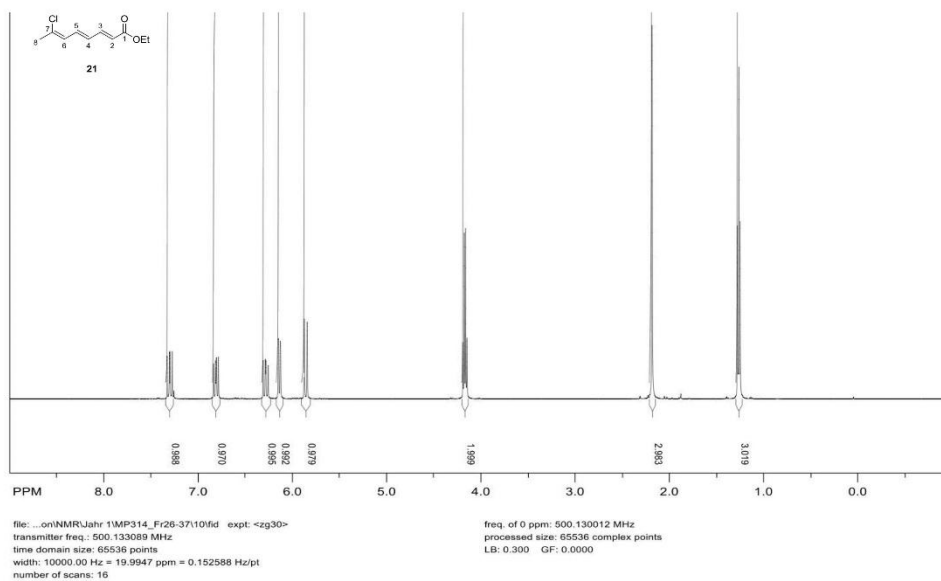
S50

¹³C NMR spectrum for (2E,4Z) 5-chloro-hexa-2,4-dien-1-ol 20 (75 MHz, CDCl₃)



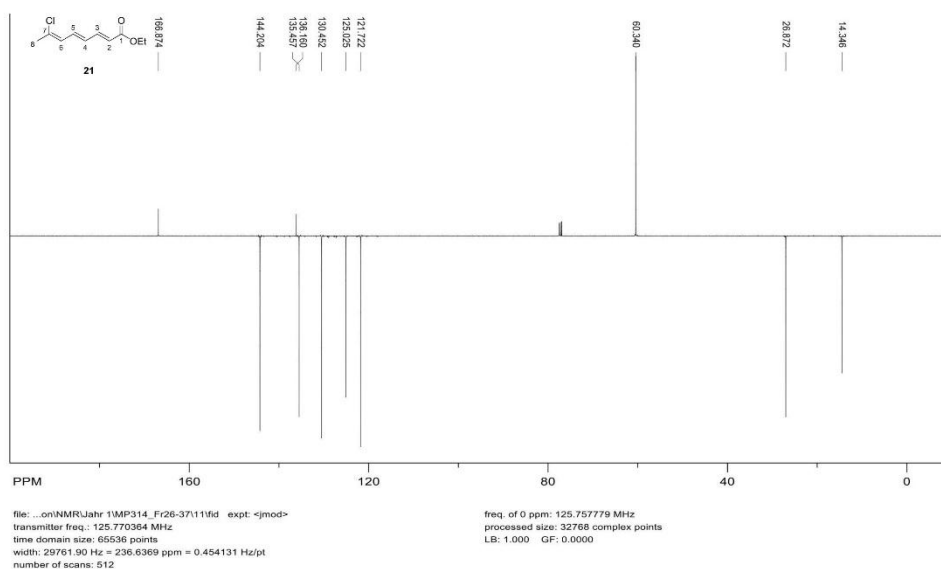
S51

¹H NMR spectrum for (2E,4E,6Z) 7-chloro-octa-2,4-trienoate 21 (500 MHz, CDCl₃)



S52

¹³C NMR spectrum for (2E,4E,6Z) ethyl-7-chloro-octa-2,4,6-trienoate 21 (125 MHz, CDCl₃)



S53

ClC1=CC=CC=C1C1=CC=CC=C1O

22

file: ...on\NMR\Jahr 1\MP316_Fr12-17\10\fid exp: <zg30>
 transmitter freq.: 500.133089 MHz
 time domain size: 65536 points
 width: 10000.00 Hz = 19.9947 ppm = 0.152588 Hz/pt
 number of scans: 16

freq. of 0 ppm: 500.130012 MHz
 processed size: 65536 complex points
 LB: 0.300 GF: 0.0000

S54

Chemical structure of compound 22 is shown above the spectrum. The structure is a substituted cyclohexene with a chlorine atom at C1, a hydroxyl group at C2, and a methyl group at C3. The numbering of the carbons is indicated in the structure.

The spectrum displays the following peak data:

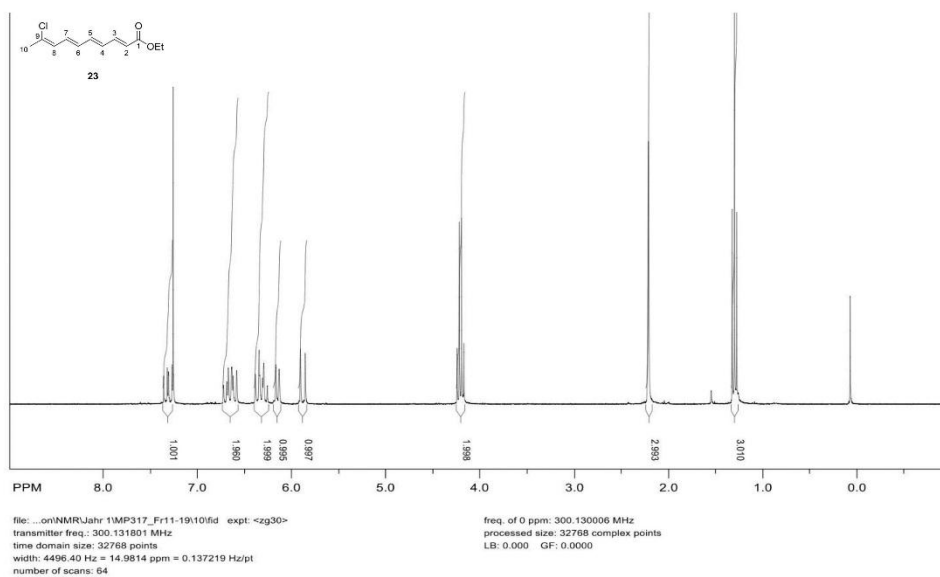
| Chemical Shift (ppm) | Assignment |
|----------------------|------------------------------|
| 135.360 | Aromatic C-OH |
| 134.461 | Aromatic C-OH |
| 132.177 | Aromatic C-OH |
| 131.873 | Aromatic C-OH |
| 77.000 | Solvent (CDCl ₃) |
| 69.553 | Aromatic C-OH |
| 26.719 | Aromatic C-OH |

File: ...onINMRJahr 1\MP316_Fr12-17\11fd exp: <jmod>
transmitter freq.: 125.770364 MHz
time domain size: 65536 points
width: 29761.90 Hz = 236.6369 ppm = 0.454131 Hz/pt
number of scans: 512

freq. of 0 ppm: 125.757769 MHz
processed size: 32768 complex points
LB: 1.000 GF: 0.0000

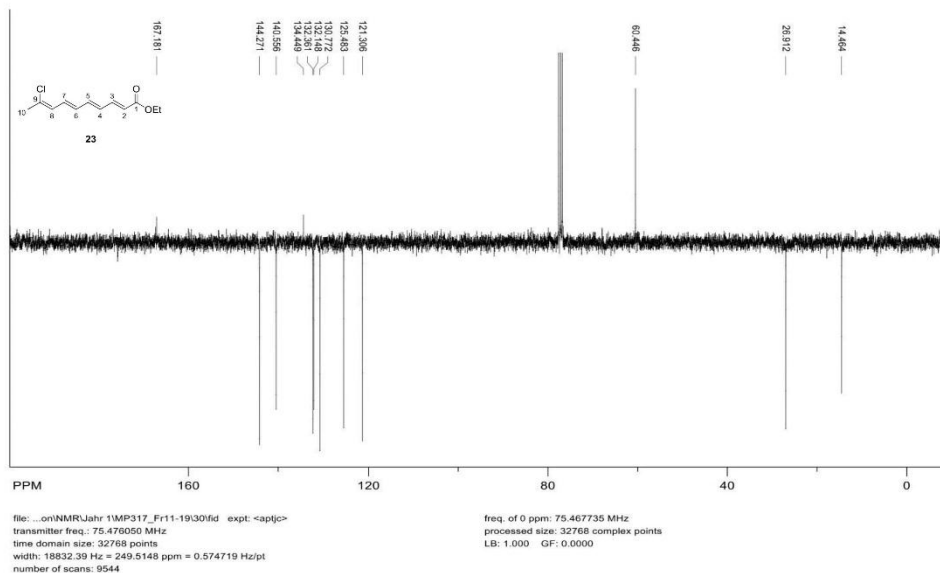
S55

¹H NMR spectrum for (2E,4E,6E,8Z) ethyl-9-chloro-deca-2,4,6,8-tetraenoate 23 (300 MHz, CDCl₃)



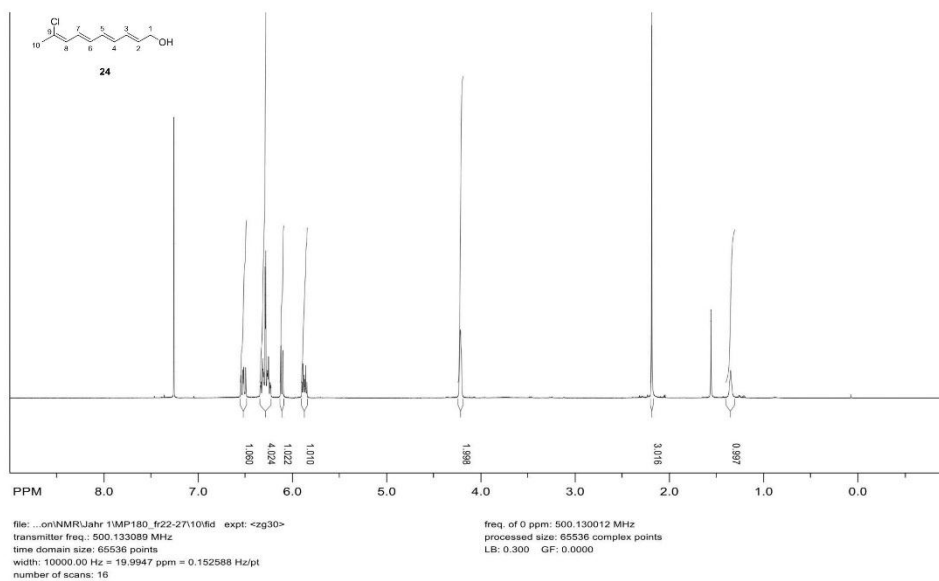
S56

¹³C NMR spectrum for (2E,4E,6E,8Z) ethyl-9-chloro-deca-2,4,6,8-tetraenoate 23 (75 MHz, CDCl₃)



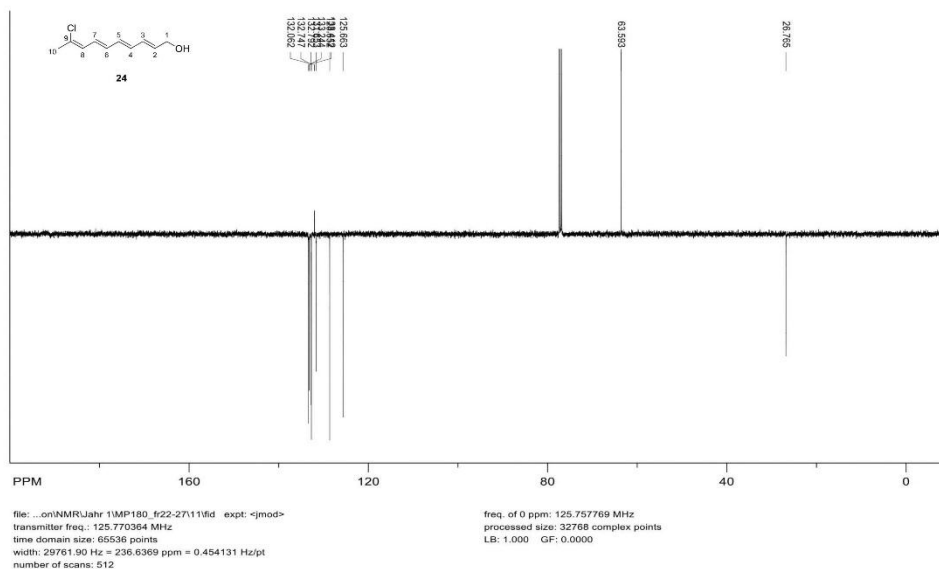
S57

¹H NMR spectrum for (2E,4E,6E,8Z) 9-chloro-deca-2,4,6,8-tetraen-1-ol 24 (500 MHz, CDCl₃)



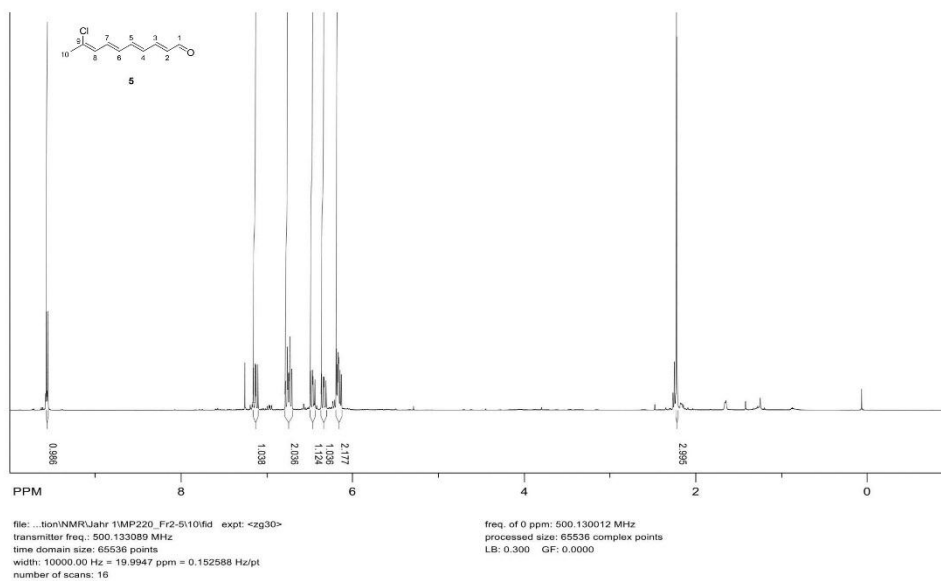
S58

¹³C NMR spectrum for (2E,4E,6E,8Z) 9-chloro-deca-2,4,6,8-tetraen-1-ol 24 (125 MHz, CDCl₃)



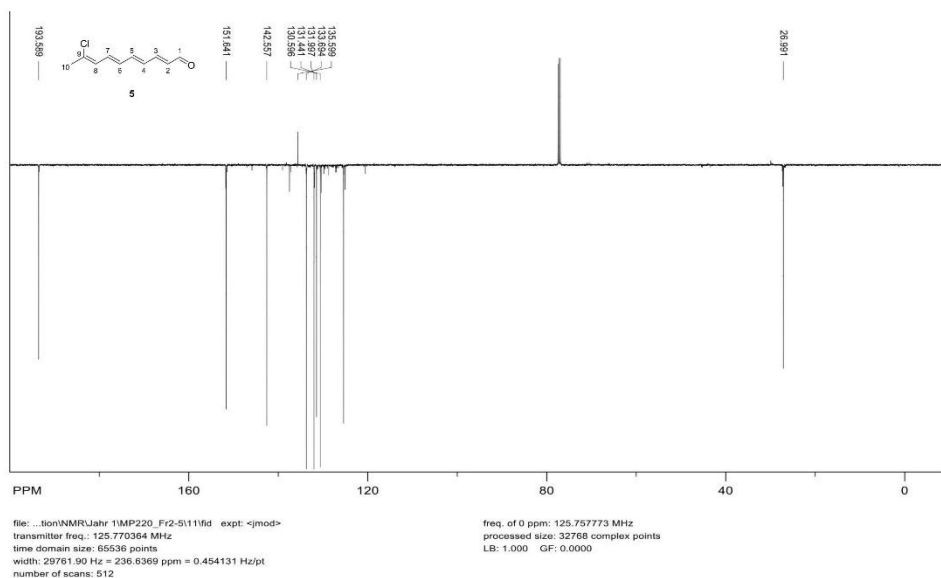
S59

¹H NMR spectrum for (2E,4E,6E,8Z) 9-chloro-deca-2,4,6,8-tetraen-1-al 5 (500 MHz, CDCl₃)



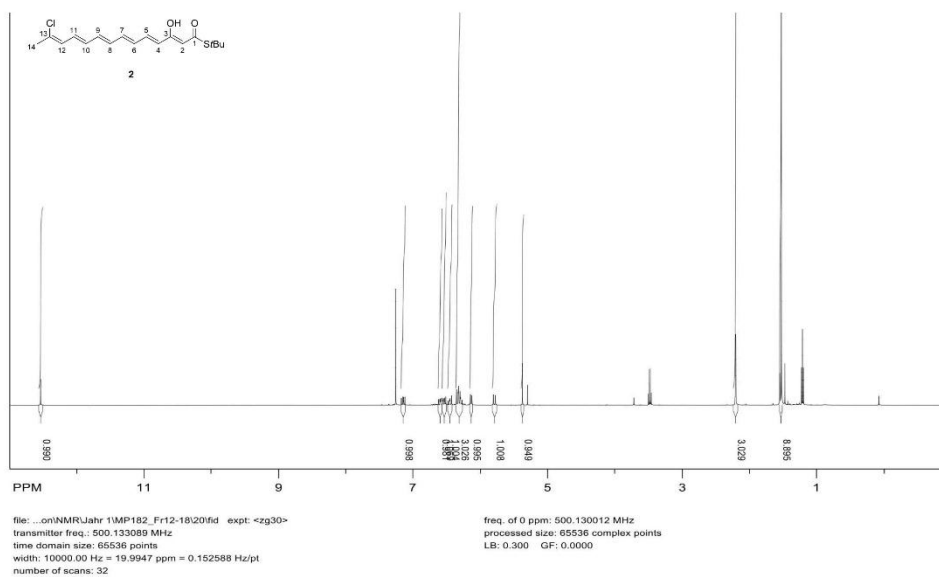
S60

¹³C NMR spectrum for (2E,4E,6E,8Z) 9-chloro-deca-2,4,6,8-tetraen-1-al 5 (125 MHz, CDCl₃)



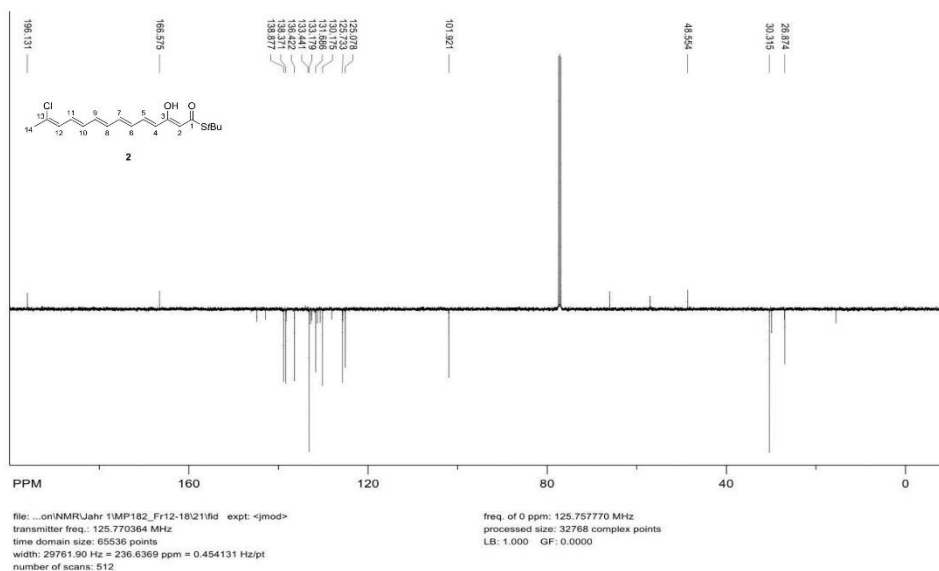
S61

¹H NMR spectrum for (2Z,4E,6E,8E,10E,12Z) S-tert-butyl-13-chloro-3hydroxy-tetradeca-2,4,6,8,10,12-hexaentioate 2 (500 MHz, CDCl₃)



S62

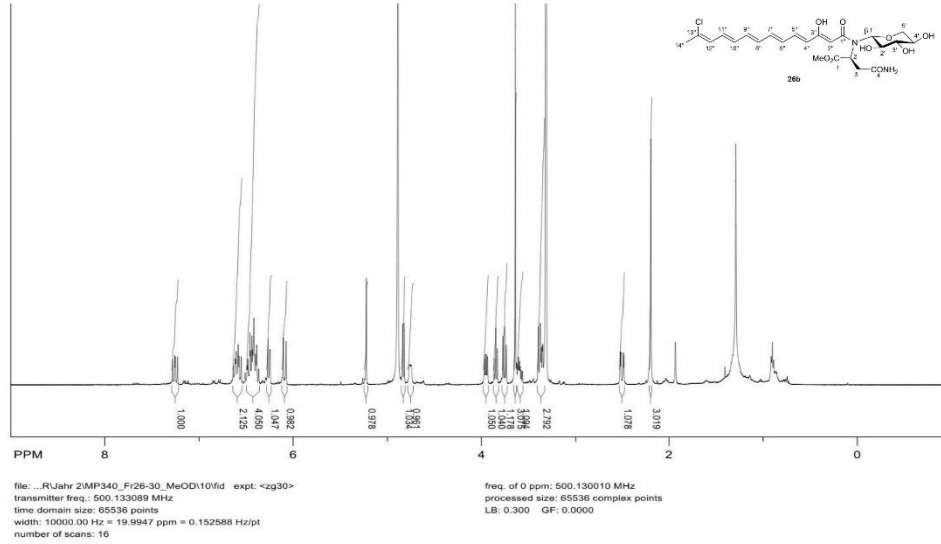
¹³C NMR spectrum for (2Z,4E,6E,8E,10E,12Z) S-tert-butyl-13-chloro-3hydroxy-tetradeca-2,4,6,8,10,12-hexaentioate 2 (125 MHz, CDCl₃)



S63

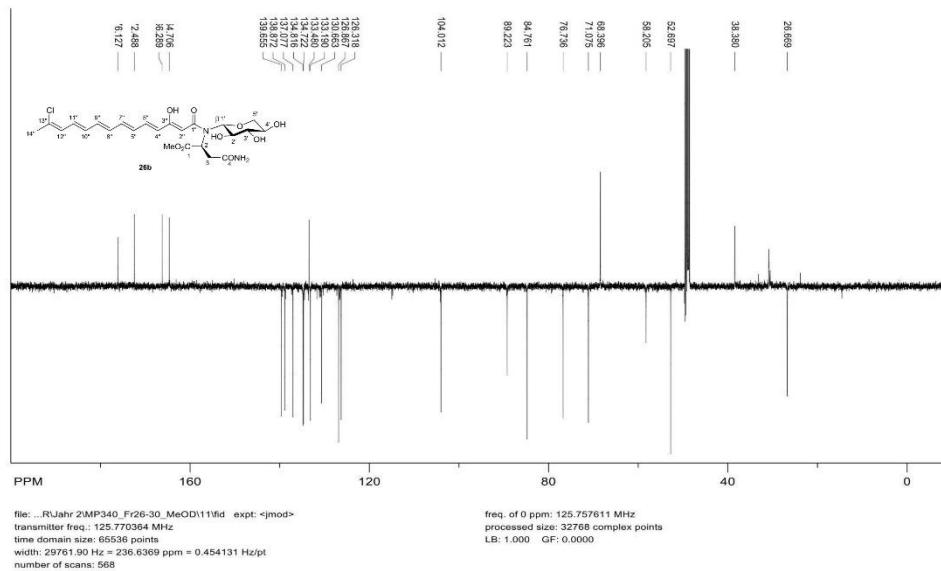
4.4. Intermediates enroute to auranoside G (1g)

¹H NMR spectrum for enol-form of methyl (2S)-(2''Z,4''E,6''E,8''E,10''E,12''Z)-N-D-xylopyranosyl)-N-(13''-chloro-1''-oxo-3''-hydroxy-tetradeca-2'',4'',6'',8'',10'',12''-hexaenyl)-2-amino-4-oxo-4-aminobutanoate 26b (500 MHz, MeOD)



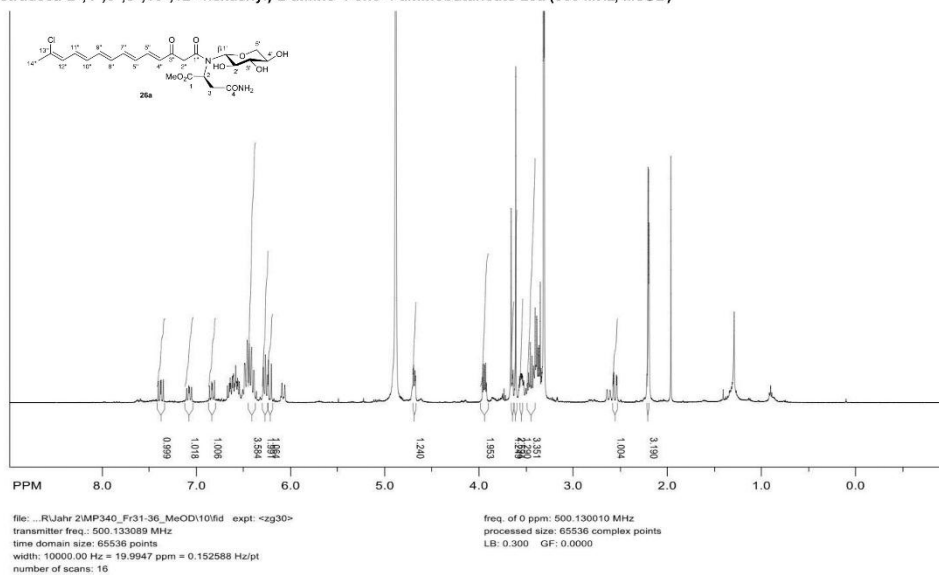
S64

¹³C NMR spectrum for enol-form of methyl (2S)-(2''Z,4''E,6''E,8''E,10''E,12''Z)-N-D-xylopyranosyl)-N-(13''-chloro-1''-oxo-3''-hydroxy-tetradeca-2'',4'',6'',8'',10'',12''-hexaenyl)-2-amino-4-oxo-4-aminobutanoate 26b (125 MHz, MeOD)



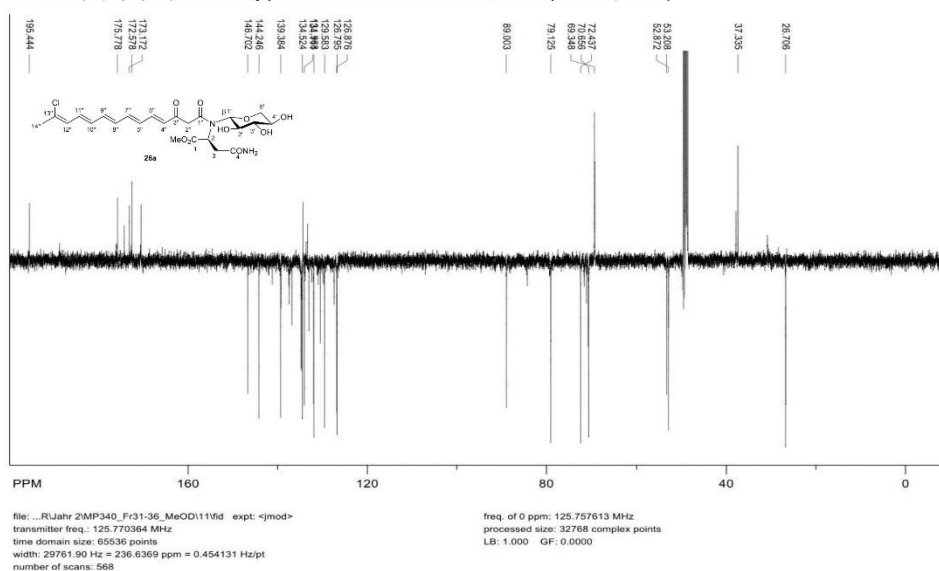
S65

¹H NMR spectrum for keto-form of methyl (2S)-(2''Z,4''E,6''E,8''E,10''E,12''Z)-β-D-xylopyranosyl)-N-(13''-chloro-1''-oxo-3''-hydroxy-tetradeca-2'',4'',6'',8'',10'',12''-hexaenyl)-2-amino-4-oxo-4-aminobutanoate 26a (500 MHz, MeOD)



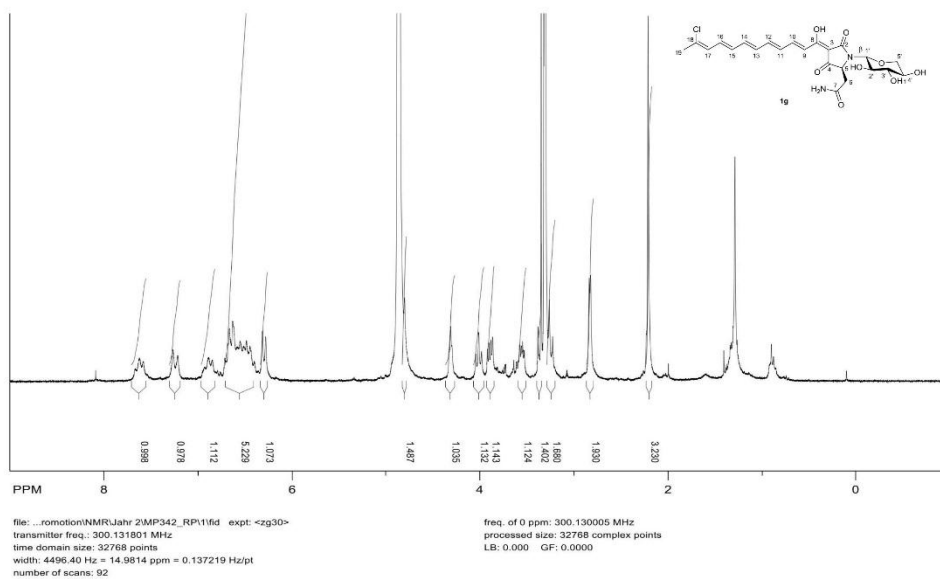
S66

¹³C NMR spectrum for keto-form of methyl (2S)-(2''Z,4''E,6''E,8''E,10''E,12''Z)-β-D-xylopyranosyl)-N-(13''-chloro-1''-oxo-3''-hydroxy-tetradeca-2'',4'',6'',8'',10'',12''-hexaenyl)-2-amino-4-oxo-4-aminobutanoate 26a (125 MHz, MeOD)



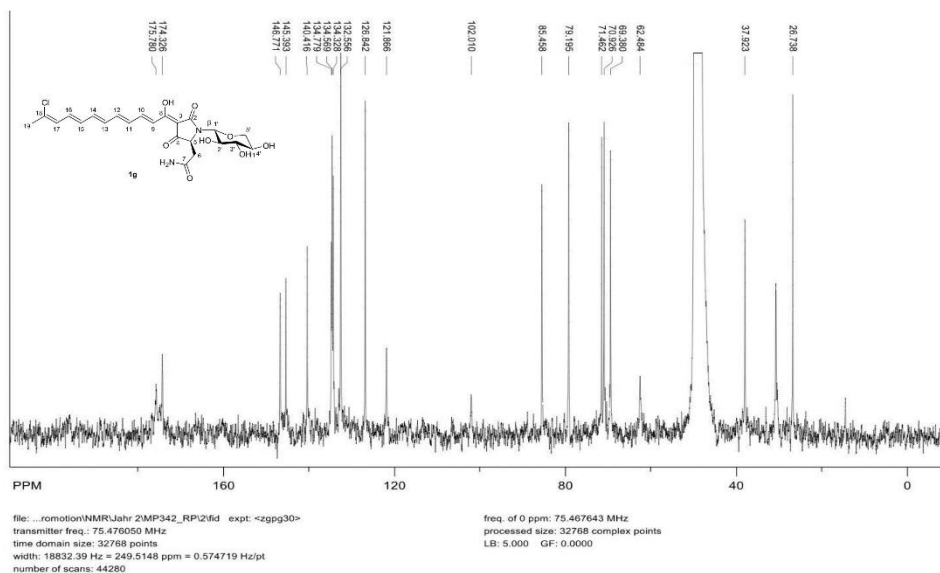
S67

¹H NMR spectrum for aurantioside G 1g (300 MHz, MeOD)



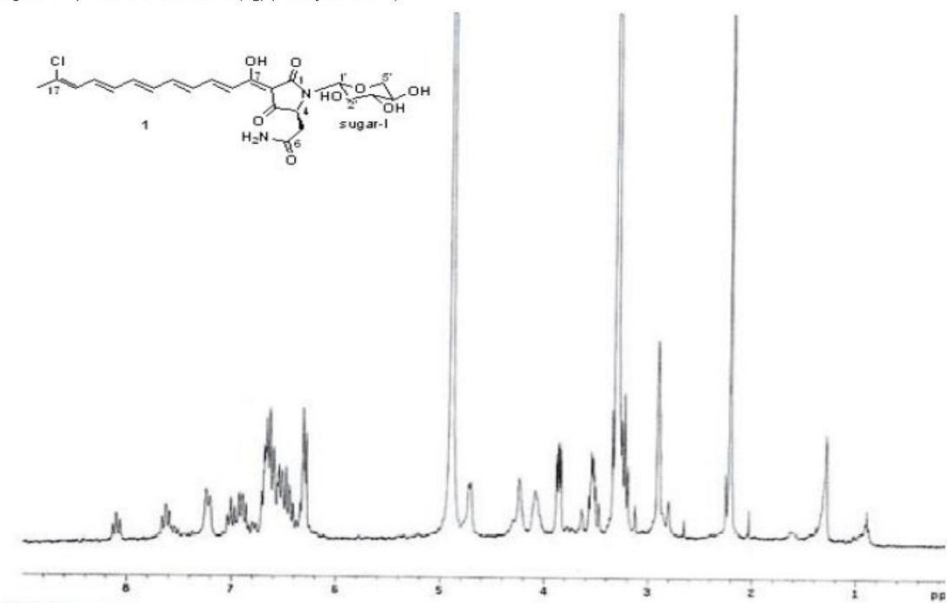
S68

¹³C NMR spectrum for aurantioside G 1g (75 MHz, MeOD)



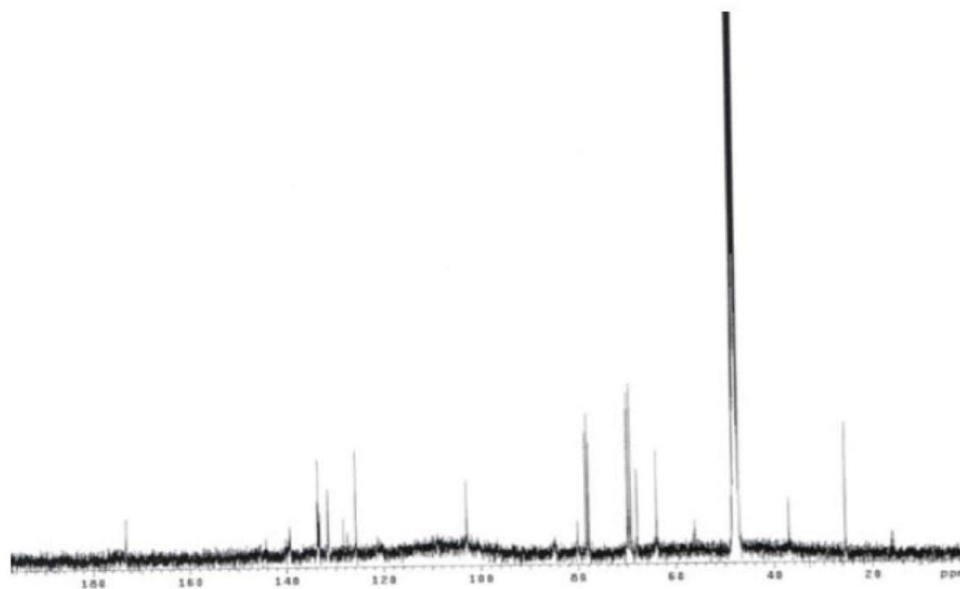
S69

Original ^1H spectra of aurantoside G (**1g**) (Ratnayake *et al.*^[8])



S70

Original ^{13}C spectra of aurantoside G (**1g**) (Ratnayake *et al.*^[8])



S71

6.2 Publikation II

Total Synthesis of the Diglycosidic Tetramic Acid Ancorinoside A

Markus Petermichl und Rainer Schobert*

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Natural Products

Total Synthesis of the Diglycosidic Tetramic Acid Ancorinoside A

Markus Petermichl and Rainer Schobert^{*,[a]}

Abstract: Ancorinoside A, a metabolite of a sponge *Ancorina* sp., was prepared in 18 steps as the first derivative of this class of glycosylated 3-acyltetramic acids. It features a β -D-glucopyranosyl-(1 \rightarrow 4)- β -D-galacturonic acid linked to a D-aspartic acid derived tetramic acid via a 3-docosanoyl spacer. The diglycoside was built up by connecting the protected monosaccharides D-galactose and D-glucose via a thioglycoside glycosylation. Attachment of the spacer by a subsequent Schmidt glycosylation of this diglycoside, TEMPO oxidation to the uronic acid, functionalisation of the spacer terminus with an *N*-(β -ketoacyl)aspartate, and a final Dieckmann cyclisation were the key steps leading to ancorinoside A. This approach should also allow access to ancorinoside D.

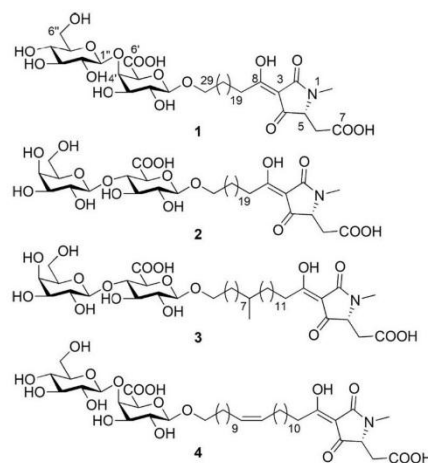
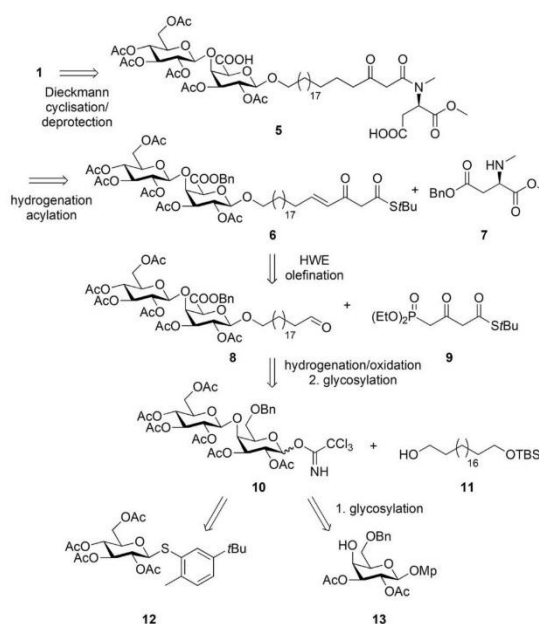


Figure 1. Structures of the ancorinosides A–D (1–4).

The ancorinosides A–D (1–4) are diglycosidic 3-acyltetramic acids with a unique dicarboxylic acid character that were isolated in 1997 by Ohta et al. (Figure 1).^[1] Their diglycosides are linked to a D-aspartic acid derived pyrrolidine-2,4-dione via a 3-docosanoyl (1–3) or a 3-tetracosenoyl spacer (4). They are combinations of D-glucose and D-galactose with the monosaccharide closer to the tetramic acid core being oxidised to the respective uronic acid which is attached to the second monosaccharide via a 4-O- β -linkage. In the case of ancorinoside A (1) the disaccharide is a 4-O- β -D-glucopyranosyl-D-galacturonic acid. Unlike the related monoglycosylated epicoccamides^[2] and most other known naturally occurring 3-acyltetramic acids, the ancorinosides are derived from a D-configured amino acid. Little is known about their biological activities. Ancorinoside A (1) was found to inhibit the blastulation of starfish (*Asterina pectinifera*) embryos and ancorinosides A–D are weak inhibitors of the matrix metalloproteinase MT1.^[1c] The magnesium salt of ancorinoside A (1) showed identical activities,^[1b] contrary to widely held assumptions on the importance of metal chelation for the bioactivity.^[3]

Scheme 1 illustrates our retrosynthetic approach to 1. A Dieckmann cyclisation of β -ketoamide precursor 5 was to yield the target compound 1 after global deprotection. Precursor 5 should be accessible by an aminolysis of thioester 6 with the

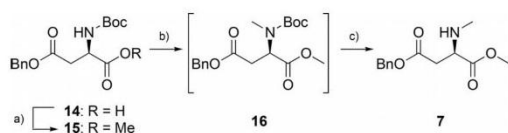
Scheme 1. Retrosynthetic approach to ancorinoside A (1). Mp = *para*-methoxyphenyl.

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Universitätsstr. 30, 95447 Bayreuth (Germany)
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Supporting information and the ORCID identification number(s) for the author(s) of this article can be found under <https://doi.org/10.1002/chem.201704379>.

N-methylated methyl *D*-aspartate **7** by Ley's protocol.^[4] Thioester **6** was to be prepared by a Horner–Wadsworth–Emmons (HWE) olefination of the fully protected aldehyde **8** with the known β -ketophosphonate **9**. Aldehyde **8** could originate, analogously to our synthesis of epicoccamide **D**,^[2c] from the coupling product of the monoprotected 1,20-eicosanediol (**11**) and diglycoside donor **10**, gone through a sequence of oxidation and deprotection steps. Imidate **10** was to be prepared by a β -selective glycosylation between thioglycoside donor **12** and the orthogonally protected *D*-galactose acceptor **13**. Both were to be synthesised starting with an anomeric protection or activation of their commercially available peracetylated β -glycosides.

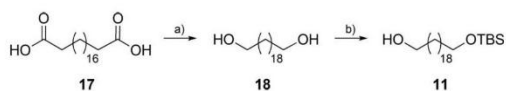
N-methylamino ester **7** was synthesised in three steps and 61 % yield from commercially available Boc-*D*-aspartic acid 4-benzylester (**14**). It was esterified with MeI to give diester **15** which was *N*-methylated affording amino ester **16**. This was not isolated but deprotected right away to leave the desired *N*-methylamino ester **7** (Scheme 2).^[5,6] A simultaneous addition



Scheme 2. Synthesis of **7**. Reagents and conditions: a) K_2CO_3 , MeI, DMF, $0^\circ C \rightarrow RT$, 1 h; 90%; b) NaH, MeI, DMF, $0^\circ C \rightarrow RT$, 1.5 h; c) TFA, CH_2Cl_2 , $0^\circ C \rightarrow RT$, 2 h; 68%.

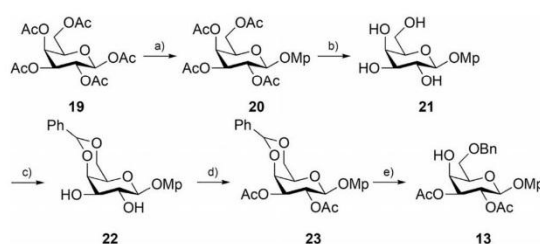
of amino ester **15** and MeI to a suspension of NaH was crucial for obtaining product **16**. It was formed as an inseparable mixture of rotamers, prone to a literature-known cyclisation to an *N*-methyl-*N*-carboxyanhydride.^[7] Thus it was immediately submitted to the next reaction step to give **7** in high yield.

The spacer precursor **11** was prepared by a desymmetrisation of the commercially available eicosanedioic acid (**17**). This was reduced to diol **18**^[8] which was mono silylated to afford **11**^[9] (Scheme 3).



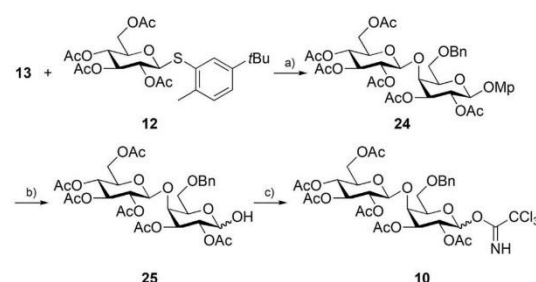
Scheme 3. Synthesis of spacer precursor **11**. Reagents and conditions: a) $LiAlH_4$, THF, $0^\circ C \rightarrow$ reflux, 16 h; 87%; b) TBSCl, imidazole, reflux, 16 h; 66%.

For the synthesis of the orthogonally protected *D*-galactose acceptor **13**, peracetylated β -*D*-galactose **19** was selectively Mp-protected at the anomeric carbon atom to furnish **20** which was subjected to a base induced Zemplén deacetylation affording **21**. This was converted to its 4,6-benzylidene acetal **22**.^[10–12] Re-acetylation of **22** gave acetal **23** which, deviating from the literature,^[13] was selectively reduced with triethylsilane and trifluoroacetic acid (TFA) to afford building block **13** in 60 % yield over five steps (Scheme 4).^[10]



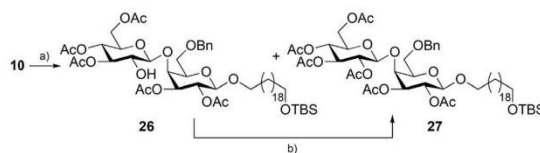
Scheme 4. Synthesis of acceptor **13**. Reagents and conditions: a) MpOH, NEt_3 , $BF_3 \cdot Et_2O$, CH_2Cl_2 , $0^\circ C \rightarrow RT$, 16 h; 98%; b) NaOMe, MeOH, RT, 15 min; 96%; c) benzaldehyde dimethyl acetal, *p*-TosOH, MeCN, RT, 1 h; 91%; d) pyridine, Ac_2O , RT, 16 h; 91%; e) Et_3SiH , TFA, CH_2Cl_2 , $0^\circ C \rightarrow RT$, 3 h; 77%.

Acceptor **13** and the known thioglycoside donor **12** reacted in a β -selective glycosylation under standard reaction conditions to give disaccharide **24** with 82 % yield.^[12,14] Surprisingly, the more reactive trichloroacetimidate donor provided disaccharide **24** in only 44 % yield. Oxidative anomeric deprotection with CAN (ceric(IV) ammonium nitrate) afforded sugar **25** which was converted to a separable mixture of α and β imidates **10a** and **10b** in 56 % overall yield (Scheme 5).



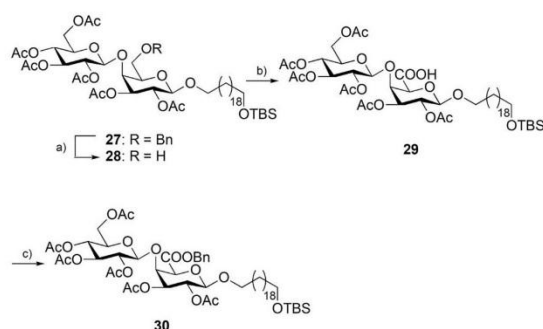
Scheme 5. Synthesis of donor **10**. Reagents and conditions: a) NIS, TfOH, MS 4 Å, CH_2Cl_2 , $-78^\circ C \rightarrow 0^\circ C$, 3 h; 82%; b) CAN, acetone/ H_2O , 3:1, RT, 30 min; 73%; c) K_2CO_3 , Cl_3CCN , $0^\circ C \rightarrow RT$, 16 h; 77%. NIS = *N*-iodosuccinimide, CAN = ceric(IV) ammonium nitrate.

The glycosylation of spacer precursor **11** with donor **10** was carried out at $-40^\circ C$ with TMSOTf as an activator. Here, an unintended partial loss of the acetyl group at the C-2'' position of the glucosyl residue was observed providing **26** as a side product. Its re-acetylation gave the desired disaccharide **27** with an overall yield of 73 % over two steps (Scheme 6).



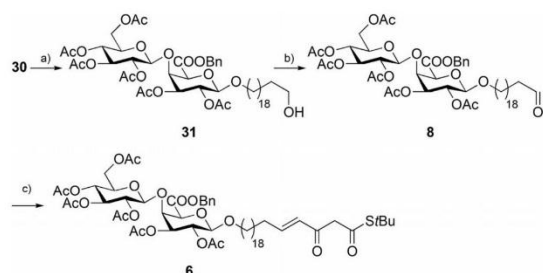
Scheme 6. Synthesis of **27**. Reagents and conditions: a) **11**, MS 4 Å, TMSOTf, CH_2Cl_2 , $-40^\circ C \rightarrow RT$, 16 h; b) pyridine, Ac_2O , $0^\circ C \rightarrow RT$, 16 h; 73%.

Disaccharide **27** was debenzylated hydrogenolytically to afford the primary alcohol **28** which was oxidised to uronic acid **29**. Its esterification gave the fully protected disaccharide building block **30** with the spacer already attached. Initially, we attempted a simultaneous oxidation–esterification sequence of alcohol **28** employing the Corey protocol^[15] which, however, left us with carboxylic acid **29**. By separating the two steps we could isolate benzyl ester **30** in good yield. Additionally, we substituted the toxic oxidant pyridinium dichromate (PDC) with an eco-friendly TEMPO/BAIB (2,2,6,6-tetramethyl-1-piperidinyloxy/bisacetoxyiodobenzene) mixture using the protocol of Epp and Widlanski^[16] (Scheme 7).



Scheme 7. Synthesis of disaccharide **30**. Reagents and conditions: a) Pd/C, H₂, EtOAc, RT, 3.5 h; 99%; b) TEMPO, BAIB, CH₂Cl₂/H₂O, RT, 2 h; 97%; c) K₂CO₃, BnBr, DMF, 0 °C → RT, 3 h; 93%. TEMPO = 2,2,6,6-tetramethyl-1-piperidinyloxy, BAIB = bisacetoxyiodobenzene.

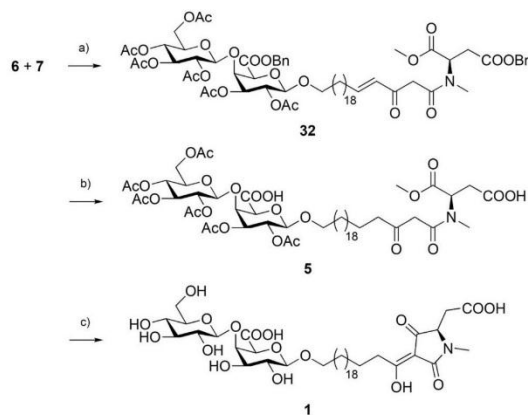
The subsequent removal of the silyl group in acetic acid afforded the primary alcohol **31** with almost quantitative yield. We abstained from using tetrabutylammonium fluoride (TBAF) in order to avoid a possible migration of acetyl protection groups and the generation of by-products.^[17] The oxidation with Dess–Martin periodinane (DMP) provided aldehyde **8** which was *E*-olefinated with Ley's β -ketophosphonate **9** in 45% yield (Scheme 8).^[2c,4,6] During this HWE reaction a time-dependent decomposition of product **6** was observed which set in prior to a complete consumption of **8** and which was



Scheme 8. Synthesis of the β -ketothioester **6**. Reagents and conditions: a) AcOH/THF/H₂O, 3:1:1, RT, 2.5 h; 92%; b) DMP, CH₂Cl₂, RT, 2 h; 79%; c) i) 9, NaH, THF, 0 °C, 1 h; ii) **8**, THF, RT, 16 h; 45%.

due to a base-induced cleavage of the 1→4 glycosidic bond of the diglycoside.

We followed Ley's protocol for the silver-mediated coupling of amino ester **7** and thioester **6** to afford β -ketoamide **32** in 63% yield when carried out under exclusion of light.^[2c,4] Since **32** was obtained as a mixture of various rotamers and tautomers, a full assignment of its NMR signals was not possible. A first attempt to cyclise it to the corresponding tetramic acid under basic Dieckmann conditions failed due to simultaneous deacetylations and transesterifications leading to a mixture of unidentified products. However, by first debenzylating diester **32** hydrogenolytically we obtained the saturated diacid **5** in 84% yield as an approximately 9:1 mixture of the keto and enol forms. A final base-induced Dieckmann cyclisation with concomitant de-acetylation gave ancorinoside A (**1**) in 48% yield as a chemically and enantiomerically pure solid after recrystallisation from methanol (Scheme 9). The NMR signals



Scheme 9. Synthesis of ancorinoside A (**1**). Reagents and conditions: a) NEt₃, AgO₂CCF₃, THF, 0 °C, 3 h; 63%; b) Pd/C, H₂, EtOAc, RT, 8 h; 84%; c) NaOMe, MeOH, RT, 1 h; 48%.

were assigned on grounds of HSQC and HMBC spectra, and by comparison with those of the natural isolate.^[1a] Our synthetic product showed an optical rotation of $[\alpha]_D^{25} = +7.0$ ($c = 0.09$, MeOH), in contrast to $[\alpha]_D^{25} = -5.5$ ($c = 0.09$, MeOH) reported for the natural isolate. This deviation might be due to impurities visible in the ¹H and ¹³C NMR spectra of the latter. The circular dichroism spectrum of our synthetic sample is in line with that reported for the natural isolate, including a positive Cotton effect ($\Delta\epsilon = +0.46$ MeOH, $\lambda = 285$ nm).^[1a] Hence, we assume that the absolute configuration proposed by Ohta et al. for the natural product is correct.

In summary, we accomplished the first total synthesis of ancorinoside A (**1**) in an overall yield of 1.6% over 18 steps in the longest linear reaction sequence. The fact that a precursor as delicate as the dicarboxylic acid **5**, featuring glycosidic bonds and potentially unprotected hydroxy groups on the sugar moiety, can be cyclised under basic Dieckmann conditions is worth mentioning. We are confident that a similar approach is

applicable also to the synthesis of the remaining ancorinosides B–D (2–4).

Conflict of interest

The authors declare no conflict of interest.

Keywords: ancorinosides • glycoconjugates • natural products • tetramic acid • total synthesis

- [1] a) S. Ohta, E. Ohta, S. Ikegami, *J. Org. Chem.* **1997**, *62*, 6452–6453; b) E. Ohta, S. Ohta, S. Ikegami, *Tetrahedron* **2001**, *57*, 4699–4703; c) M. Fujita, Y. Nakao, S. Matsunaga, M. Seiki, Y. Itoh, R. W. M. van Soest, N. Fusetani, *Tetrahedron* **2001**, *57*, 1229–1234.
- [2] a) A. D. Wright, C. Osterhage, G. M. König, *Org. Biomol. Chem.* **2003**, *1*, 507–510; b) H. V. K. Wangun, H.-M. Dahse, C. Hertweck, *J. Nat. Prod.* **2007**, *70*, 1800–1803; c) S. Loscher, R. Schobert, *Chem. Eur. J.* **2013**, *19*, 10619–10624.
- [3] a) M. Petrolig, O. Igglessi-Markopoulou, J. Markopoulos, *Heterocycl. Commun.* **2000**, *6*, 157–164; b) M. Zaghouani, B. Nay, *Nat. Prod. Rep.* **2016**, *33*, 540–548; c) B. Biersack, R. Diestel, C. Jagusch, F. Sasse, R. Schobert, *J. Inorg. Biochem.* **2009**, *103*, 72–76.
- [4] S. V. Ley, S. C. Smith, P. R. Woodward, *Tetrahedron* **1992**, *48*, 1145–1174.
- [5] a) E. A. Englund, H. N. Gopi, D. H. Appella, *Org. Lett.* **2004**, *6*, 213–215; b) M. Petermichl, S. Loscher, R. Schobert, *Angew. Chem. Int. Ed.* **2016**, *55*, 10122–10125; *Angew. Chem.* **2016**, *128*, 10276–10279.
- [6] O. Hartmann, M. Kalesse, *Angew. Chem. Int. Ed.* **2014**, *53*, 7335–7338; *Angew. Chem.* **2014**, *126*, 7463–7466.
- [7] E. Frérot, J. Coste, J. Poncet, P. Jouin, B. Castro, *Tetrahedron Lett.* **1992**, *33*, 2815–2816.
- [8] Y. Gao, J. Z. Vlahakis, W. A. Szarek, I. Brockhausen, *Bioorg. Med. Chem.* **2013**, *21*, 1305–1311.
- [9] T. Suzuki, T. Yamamoto, Y. Tezuka, *J. Am. Chem. Soc.* **2014**, *136*, 10148–10155.
- [10] J. Li, W. Li, B. Yu, *Org. Biomol. Chem.* **2013**, *11*, 4971–4974.
- [11] J. M. Pons, A. Dumont, G. Sautejeau, E. Fugier, A. Baron, S. Dukan, B. Vauzeilles, *Angew. Chem. Int. Ed.* **2014**, *53*, 1275–1278; *Angew. Chem.* **2014**, *126*, 1299–1302.
- [12] Z. Zhang, G. Magnusson, *J. Org. Chem.* **1996**, *61*, 2383–2393.
- [13] M. P. DeNinno, J. B. Etienne, K. C. Duplantier, *Tetrahedron Lett.* **1995**, *36*, 669–672.
- [14] R. Autar, R. M. J. Liskamp, R. J. Pieters, *Carbohydr. Res.* **2005**, *340*, 2436–2442.
- [15] E. J. Corey, B. Samuelsson, *J. Org. Chem.* **1984**, *49*, 4735–4735.
- [16] a) J. B. Epp, T. S. Widlanski, *J. Org. Chem.* **1999**, *64*, 293–295; b) C. Cai, D. M. Dickinson, L. Li, S. Masuko, M. Suflita, V. Schultz, S. D. Nelson, U. Bhaskar, J. Liu, R. J. Linhardt, *Org. Lett.* **2014**, *16*, 2240–2243; c) L. J. van den Bos, J. D. C. Codée, J. C. van der Toorn, T. J. Boltje, J. H. van Boom, H. S. Overkleef, G. A. van der Marel, *Org. Lett.* **2004**, *6*, 2165–2168.
- [17] O. P. Chevallier, M. E. Migaud, *Beilstein J. Org. Chem.* **2006**, *2*, 14.

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Supporting Information

Total Synthesis of the Diglycosidic Tetramic Acid Ancorinoside A

Markus Petermichl and Rainer Schobert^{*,[a]}

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General

IR spectra were recorded with an FT-IR spectrophotometer equipped with an ATR unit. ^1H -NMR, ^{13}C -NMR and were obtained using a Bruker DRX 500 spectrometer. Chemical shifts are given in parts per million using the residual solvent peak as an internal standard acc. to Gottlieb, 7.26 ppm (proton) and 77.16 ppm (carbon) for CDCl_3 and 3.31 ppm (proton) and 49.00 (carbon) for $\text{MeOD}-d_4$. Coupling constants (J) are quoted in Hz. Multiplicity abbreviation used: s singlet, d doublet, t triplet, q quintette, and m multiplet. High resolution mass spectra were obtained with a UPLC/Orbitrap MS system in ESI mode. Optical rotations were measured at 589 nm (Na-D line) on a PerkinElmer 241 Polarimeter using solutions in chloroform and methanol. For chromatography silica gel 60 (230-400 mesh) was used. All reagents were purchased from commercial sources and were used without further purification. All anhydrous solvents were used as supplied, except tetrahydrofuran and dichloromethane which were freshly distilled according to standard procedures. Reactions were routinely carried out under an argon atmosphere unless stated otherwise. All glassware was flame-dried before use.

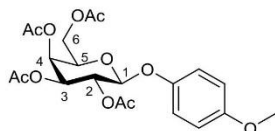
Chromatography: Analytical thin layer chromatography (TLC) was carried out using Merck Kieselgel 60 F_{254} pre-coated aluminium-backed foil plates. The compounds were visualized with UV light (254 nm or 360 nm) and/or ceric ammonium molybdate (CAM).

Flash chromatography was performed at medium pressure using Marchery-Nagel silica gel 60, pore size 40 – 63 μm with the eluent specified.

Analytical HPLC measurements were performed on a Beckman System Gold Programmable Solvent Module 126 using a Phenomenex Kinetex® C-18-HPLC column, length 250 x 4.6 mm, pore size 100 Å, particle size 5 μm . Detection by a Beckman Instruments Diode Array Detection Module 168.

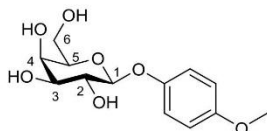
Experimental procedures

para-Methoxyphenyl-2,3,4,6-tetra-*O*-acetyl-1-*O*- β -D-galactopyranoside (**20**)



A solution of 3.903 g (10.0 mmol, 1.00 eq) 1,2,3,4,6-penta-*O*- β -D-galactopyranoside (**19**), 1.490 g (12.0 mmol, 1.20 eq) *para*-methoxyphenol and 0.69 mL (5.0 mmol, 0.50 eq) NEt₃ in 33 mL dry CH₂Cl₂ at 0 °C was treated dropwise with 1.90 mL (15.0 mmol, 1.50 eq) BF₃ etherate. The mixture was allowed to warm to room temperature and stirred at this temperature for 16 h. 60 mL of a 1 M aqueous NaOH solution were added at 0 °C and stirring was continued for a further 30 min. The aqueous phase was separated and extracted three times with CH₂Cl₂. The combined organic phases were washed with water, dried over Na₂SO₄ and concentrated in vacuo. The remaining yellow oil was purified by column chromatography (silica, 70/30 cHex/EtOAc) to afford the title compound as a colourless solid: 4.459 g (9.812 mmol, 98%). *m*_p = 100–101 °C; [α]_D²⁵ +2.4 ° (*c* = 1.00, CHCl₃); *R*_f = 0.45 (1:1 cHex/EtOAc); ¹H NMR (500 MHz, CDCl₃) δ 6.98–6.92 (m, 2H; Ar-H), 6.84–6.79 (m, 2H; Ar-H), 5.45 (dd, *J*=8.1, 10.5 Hz, 1H; 2-H), 5.45–5.43 (m, 1H; 4-H), 5.08 (dd, *J*=3.5, 10.5 Hz, 1H; 3-H), 4.91 (d, *J*=8.1 Hz, 1H; 1-H), 4.23 (dd, *J*=6.9, 11.3 Hz, 1H; 6-H^a), 4.15 (dd, *J*=6.5, 11.3 Hz, 1H; 6-H^b), 4.00 (ddd, *J*=0.9, 6.5, 6.9 Hz, 1H; 5-H), 3.77 (s, 3H; OMe), 2.18 (s, 3H; CH₃-Ac), 2.08 (s, 3H; CH₃-Ac), 2.05 (s, 3H; CH₃-Ac), 2.00 ppm (s, 3H; CH₃-Ac); ¹³C NMR (125 MHz, CDCl₃) δ 170.5, 170.4, 170.3, 169.5, 155.9, 151.1, 118.7, 114.7, 101.0, 71.03, 70.99, 68.9, 67.0, 61.4, 55.8, 20.9, 20.82, 20.81, 20.7 ppm; IR ν [cm⁻¹] = 1744(s), 1507(s), 1442(w), 1368(m), 1209(s), 1166(w), 1126(w), 1072(s), 1043(s), 955(w), 914(m), 830(m), 757(w), 731(m). All data in accordance with literature.^[1]

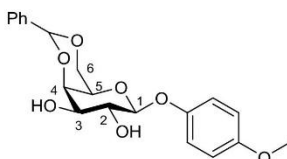
para-Methoxyphenyl-1-*O*- β -D-galactopyranoside (**21**)



A solution of 4.466 g (9.826 mmol, 1.00 eq) **20** in 66 mL methanol at room temperature was treated with 0.265 g (4.913 mmol, 0.50 eq) NaOMe in one portion. The reaction was stirred for 15 min at this temperature. Then the solution was acidified by adding DOWEX 50WX8-100® resin. The mixture was filtrated and the solvent evaporated in vacuo to afford the title compound as a colourless solid: 2.702 g (9.438 mmol, 96%). *m*_p = 157 °C; [α]_D²⁵ –36.8 ° (*c* = 1.00, H₂O); ¹H NMR (500 MHz, MeOD) δ 7.08–

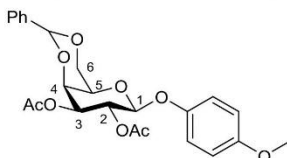
7.03 (m, 2H; Ar-H), 6.86-6.80 (m, 2H; Ar-H), 4.73 (d, $J=7.8$ Hz, 1H; 1-H), 3.89 (dd, $J=0.9, 3.3$ Hz, 1H; 4-H), 3.78-3.71 (m, 3H; 2-H, 6-H), 3.74 (s, 3H; OMe), 3.64 (ddd, $J=0.9, 5.2, 6.9$ Hz, 1H; 5-H), 3.56 ppm (dd, $J=3.3, 9.7$ Hz, 1H; 3-H); ^{13}C NMR (125 MHz, MeOD) δ 156.5, 153.3, 119.2, 115.4, 104.0, 76.9, 74.8, 72.3, 70.2, 62.4, 56.0 ppm; IR $\nu[\text{cm}^{-1}]$ = 3374 (br m), 1639 (w), 1508 (s), 1460 (w), 1441 (w), 1398 (w), 1296 (w), 1218 (s), 1142 (m), 1071 (s), 1041 (s), 828 (m), 743 (m). All data in accordance with literature.^[2]

***para*-Methoxyphenyl-4,6-*O*-benzylidene-1-*O*- β -D-galactopyranoside (**22**)**



To a suspension of 2.702 g (9.438 mmol, 1.00 eq) **21** in 50 mL dry MeCN at room temperature were added 2.83 mL (18.877 mmol, 2.00 eq) benzaldehyde dimethyl acetal and 0.090 g (0.472 mmol, 0.05 eq) *p*-toluenesulfonic acid. The reaction was stirred vigorously for 1 h and then cooled to 0 °C. The precipitated solid was collected and the filtrate concentrated in vacuo. The residue was suspended in methanol and the combined solids collected to afford the title compound as a white solid: 3.224 g (8.611 mmol, 91%). m_p = 230 °C; $[\alpha]_D^{25}$ -87.9 ° (c = 1.00, MeOH/ CHCl_3 1:1); R_f = 0.44 (95:5 $\text{CH}_2\text{Cl}_2/\text{MeOH}$); ^1H NMR (500 MHz, CDCl_3) δ 7.54-7.50 (m, 2H; Ar-H), 7.40-7.36 (m, 3H; Ar-H), 7.09-7.04 (m, 2H; Ar-H), 6.85-6.80 (m, 2H; Ar-H), 5.58 (s, 1H; CH-Ph), 4.79 (d, $J=7.9$ Hz, 1H; 1-H), 4.37 (dd, $J=0.9, 12.6$ Hz, 1H; 6-H^a), 4.28 (d, $J=3.6$ Hz, 1H; 4-H), 4.11 (dd, $J=1.6, 12.6$ Hz, 1H; 6-H^b), 4.01 (dd, $J=7.9, 9.6$ Hz, 1H; 2-H), 3.79-3.75 (m, 1H; 3-H), 3.78 (s, 3H; OMe), 3.59 (br s, 1H; 5-H), 2.57 (d, $J=2.1$ Hz, 1H; OH), 2.06 ppm (br s, 1H; OH); ^{13}C NMR (125 MHz, CDCl_3) δ 155.7, 151.2, 137.5, 129.5, 128.4, 126.6, 119.2, 114.6, 102.5, 101.7, 75.2, 72.8, 71.6, 69.2, 66.9, 55.8 ppm; IR $\nu[\text{cm}^{-1}]$ = 3395 (br w), 2883 (w), 1508 (s), 1453 (w), 1401 (w), 1365 (w), 1216 (s), 1173 (w), 1103 (m), 1077 (s), 1058 (s), 1036 (s), 998 (m), 979 (m), 902 (w), 871 (w), 824 (m), 747 (m), 734 (m), 697 (m). All data in accordance with literature.^[3]

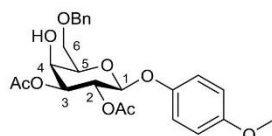
***para*-Methoxyphenyl-2,3-di-*O*-acetyl-4,6-benzylidene-1-*O*- β -D-galactopyranoside (**23**)**



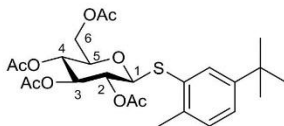
To a solution of 2.645 g (7.065 mmol, 1.00 eq) **22** in 25 mL pyridine at room temperature were added 2.00 mL (21.195 mmol, 3.00 eq) Ac_2O . The reaction was stirred for 16 h at this temperature. Then a saturated aqueous NaHCO_3 solution was added. The aqueous phase was separated and extracted

two times with EtOAc. The combined organic phases were washed with brine, dried over Na₂SO₄ and concentrated in vacuo to afford the title compound as a colourless solid: 2.955 g (6.444 mmol, 91%). $m_p = 189\text{ }^{\circ}\text{C}$; $[\alpha]^{25}_{\text{D}} +34.9\text{ }^{\circ}$ ($c = 1.00$, CHCl₃); $R_f = 0.44$ (1:1 cHex/EtOAc); ¹H NMR (500 MHz, CDCl₃) δ 7.56-7.51 (m, 2H; Ar-H), 7.41-7.34 (m, 3H; Ar-H), 7.02-6.97 (m, 2H; Ar-H), 6.84-6.78 (m, 2H; Ar-H), 5.62 (dd, $J=8.0, 10.5$ Hz, 1H; 2-H), 5.53 (s, 1H; CH-Ph), 5.03 (dd, $J=3.7, 10.5$ Hz, 1H; 3-H), 4.96 (d, $J=8.0$ Hz, 1H; 1-H), 4.43 (dd, $J=0.7, 3.7$ Hz, 1H; 4-H), 4.37 (dd, $J=1.6, 12.5$ Hz, 1H; 6-H^a), 4.09 (dd, $J=1.7, 12.5$ Hz, 1H; 6-H^b), 3.77 (s, 3H; OMe), 3.60 (dd, $J=1.6, 1.7$ Hz, 1H; 5-H), 2.09 (s, 3H; CH₃-Ac), 2.08 ppm (s, 3H; CH₃-Ac); ¹³C NMR (125 MHz, CDCl₃) δ 171.0, 169.4, 155.7, 151.4, 137.5, 129.3, 128.3, 126.6, 119.2, 114.6, 101.3, 101.2, 73.3, 72.1, 69.0, 68.6, 66.6, 55.8, 21.1, 21.0 ppm; IR $\nu[\text{cm}^{-1}] = 2916$ (br w), 1747 (m), 1506 (s), 1454 (w), 1405 (w), 1369 (m), 1212 (s), 1179 (m), 1133 (w), 1083 (m), 1061 (s), 1036 (s), 997 (m), 909 (m), 828 (m), 730 (s), 698 (m). All data in accordance with literature.^[1]

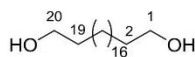
***para*-Methoxyphenyl-2,3-di-O-acetyl-6-O-benzyl-1-O- β -D-galactopyranoside (13)**



A mixture of 0.458 g (1.00 mmol, 1.00 eq) **23**, 0.50 g freshly activated molecular sieves (4 Å), and 5 mL dry CH₂Cl₂ was slowly treated with 0.80 mL (5.00 mmol, 5.00 eq) triethylsilane and 0.57 mL (5.00 mmol, 5.00 eq) TFA. The reaction was monitored by TLC. After consumption of the entire starting material the reaction mixture was filtered through a plug of Celite® and the filtrate was washed with a saturated aqueous solution of NaHCO₃. The aqueous phase was extracted three times with EtOAc. The combined organic phases were washed with brine, dried over Na₂SO₄ and concentrated in vacuo. The yellow oily residue was purified by column chromatography (silica, 70/30 cHex/EtOAc) to afford the title compound as a colourless solid: 0.354 g (0.769 mmol, 77%). $m_p = 113.2\text{ }^{\circ}\text{C}$; $[\alpha]^{25}_{\text{D}} +4.71\text{ }^{\circ}$ ($c = 1.00$, CHCl₃); $R_f = 0.42$ (1:1 cHex/EtOAc); ¹H NMR (500 MHz, CDCl₃) δ 7.36-7.26 (m, 5H; Ar-H), 7.02-6.95 (m, 2H; Ar-H), 6.83-6.77 (m, 2H; Ar-H), 5.54 (dd, $J=7.9, 10.3$ Hz, 1H; 2-H), 5.02 (dd, $J=3.2, 10.3$ Hz, 1H; 3-H), 4.93 (d, $J=7.9$ Hz, 1H; 1-H), 4.58 (d, $J=12.0$ Hz, 1H; Ph-CH^aH^b), 4.55 (d, $J=12.0$ Hz, 1H; Ph-CH^aH^b), 4.20 (br d, $J=2.4$ Hz, 1H; 4-H), 3.85-3.78 (m, 3H; 5-H, 6-H), 3.76 (s, 3H; OMe), 3.09 (br s, 1H; OH), 2.11 (s, 3H; CH₃-Ac), 2.08 ppm (s, 3H; CH₃-Ac); ¹³C NMR (125 MHz, CDCl₃) δ 170.4, 169.7, 155.5, 151.2, 137.7, 128.5, 127.9, 127.7, 118.6, 114.5, 100.8, 73.7, 73.6, 73.5, 69.3, 69.2, 67.8, 55.6, 20.9, 20.8 ppm; IR $\nu[\text{cm}^{-1}] = 3461$ (br w), 2932 (br w), 1748 (s), 1507 (s), 1455 (w), 1368 (m), 1213 (s), 1157 (w), 1058 (s), 909 (m), 829 (m), 730 (s), 699 (s). All data in accordance with literature.^[1]

(2-Methyl-5-*tert*-butyl-phenyl)-2,3,4,6-tetra-O-acetyl-1-S- β -D-thio-glucopyranoside (12)

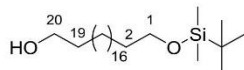
A solution of 3.904 g (10.0 mmol, 1.00 eq) 1,2,3,4,6-penta-O-acetyl- β -D-glucopyranoside and 2.20 mL (12.0 mmol, 1.20 eq) 2-methyl-5-*tert*-butyl-thiophenol at 0 °C in 20 mL dry CH_2Cl_2 was treated dropwise with 1.72 mL (14.0 mmol, 1.40 eq) BF_3 etherate. The reaction mixture was slowly warmed to room temperature and was stirred at this temperature for 16 h. 80 mL of a 1 M aqueous NaOH solution were added and the resulting mixture was stirred for a further 30 min. The aqueous phase was separated and extracted three times with CH_2Cl_2 . The combined organic phases were washed with H_2O , dried over Na_2SO_4 and concentrated in vacuo. The crude pinkish oil was purified by column chromatography (silica, 70/30 cHex/EtOAc) to afford the title compound as a colourless syrup: 4.687 g (9.179 mmol, 92%). $[\alpha]_{\text{D}}^{25} -8.3^\circ$ ($c = 1.00$, CHCl_3); $R_f = 0.23$ (3:1 cHex/EtOAc); ^1H NMR (500 MHz, CDCl_3) δ 7.53 (d, $J=2.0$ Hz, 1H; Ar-H), 7.24 (dd, $J=2.0, 8.0$ Hz, 1H; Ar-H), 7.13 (d, $J=8.0$ Hz, 1H; Ar-H), 5.21 (dd, $J=9.4, 9.7$ Hz, 1H; 3-H), 5.08 (dd, $J=9.7, 10.0$ Hz, 1H; 4-H), 5.03 (dd, $J=9.4, 10.1$ Hz, 1H; 2-H), 4.66 (d, $J=10.1$ Hz, 1H; 1-H), 4.25 (dd, $J=4.9, 12.3$ Hz, 1H; 6-H^a), 4.11 (dd, $J=2.2, 12.3$ Hz, 1H; 6-H^b), 3.69 (ddd, $J=2.2, 4.9, 10.0$ Hz, 1H; 5-H), 2.35 (s, 3H; CH_3), 2.07 (s, 3H; $\text{CH}_3\text{-Ac}$), 2.05 (s, 3H; $\text{CH}_3\text{-Ac}$), 2.00 (s, 3H; $\text{CH}_3\text{-Ac}$), 1.98 (s, 3H; $\text{CH}_3\text{-Ac}$), 1.30 ppm (s, 9H; *t*Bu); ^{13}C NMR (125 MHz, CDCl_3) δ 170.7, 170.2, 169.4, 169.3, 149.7, 137.4, 131.4, 130.4, 130.1, 125.7, 86.9, 75.8, 74.0, 70.1, 68.1, 62.3, 31.3, 20.79, 20.78, 20.65, 20.61, 20.4 ppm; IR $\nu[\text{cm}^{-1}] = 2962$ (w), 1748 (s), 1489 (w), 1436 (w), 1213 (s), 1120 (w), 1088 (s), 1034 (s), 957 (w), 911 (m), 824 (m), 730 (m), 683 (w). All data in accordance with literature.^[4]

Eicosan-1,20-diol (18)

A suspension of 0.855 g (2.50 mmol, 1.00 eq) eicosanedioic acid in 60 mL dry THF at 0 °C was treated with 0.356 g (9.375 mmol, 3.75 eq) LiAlH_4 in several portions and then heated under reflux while stirring for 16 h. The reaction was stopped by adding 100 mL of a 33 wt% aqueous citric acid. The aqueous phase was separated and extracted three times with CH_2Cl_2 . The combined organic phases were washed with H_2O , dried over Na_2SO_4 and concentrated in vacuo to afford the title compound as a white solid: 0.681 g (2.165 mmol, 87%). $m_p = 99^\circ\text{C}$; $R_f = 0.52$ (9:1 $\text{CH}_2\text{Cl}_2/\text{MeOH}$); ^1H NMR (500 MHz, CDCl_3) δ 3.64 (t, $J=6.7$ Hz, 4H; 1-H, 20-H), 1.56 (qui, $J=6.7$ Hz, 4H; 2-H, 19-H), 1.35-1.23 ppm (m, 32H; 3-H to 18-H); ^{13}C NMR (125 MHz, CDCl_3) δ 63.3, 33.0, 29.82, 29.80, 29.76, 29.75, 29.6, 25.9 ppm; IR $\nu[\text{cm}^{-1}] = 3254$ (br m), 2918 (s), 2849 (s), 1732 (br w), 1473 (m), 1462 (m), 1409

(w), 1355 (w), 1123 (w), 1060 (m), 1027 (m), 1001 (m), 948 (w), 921 (w), 893 (w), 732 (m), 719 (m). All data in accordance with literature.^[5]

1-((*tert*-Butyldimethylsilyl)oxy)-20-eicosandiol (**11**)



To a solution of 0.363 g (1.154 mmol, 1.00 eq) **18** in 20 mL dry THF at 60 °C were added 0.217 g (1.443 mmol, 1.25 eq) TBSCl and 0.232 g (3.404 mmol, 2.95 eq) imidazole. The suspension was heated to reflux and stirred at this temperature for 16 h. Then the reaction was diluted with EtOAc and saturated aqueous NaHCO₃ was added. The aqueous phase was separated and extracted three times with EtOAc. The combined organic phases were dried over Na₂SO₄ and concentrated in vacuo. The remaining colourless waxy solid was purified by column chromatography (silica, 85/15 cHex/EtOAc) to afford the title compound as a colourless waxy solid: 0.206 g (0.480 mmol, 66%, according to recovered starting material). *m*_p = not detectable; *R*_f = 0.14 (9:1 cHex/EtOAc); ¹H NMR (500 MHz, CDCl₃) δ 3.64 (t, *J*=6.8 Hz, 2H; 20-H), 3.59 (t, *J*=6.7 Hz, 2H; 1-H), 1.56 (qui, *J*=6.8 Hz, 2H; 19-H), 1.50 (qui, *J*=6.8 Hz, 2H; 2-H), 1.35-1.23 (m, 32H; 3-H to 18-H), 0.89 (s, 9H; *t*Bu), 0.05 ppm (s, 6H; CH₃-Si); ¹³C NMR (125 MHz, CDCl₃) δ 63.5, 63.3, 33.0, 32.9, 29.8, 29.6, 26.1, 26.0, 25.9 ppm; IR ν[cm⁻¹] = 3334 (br w), 2923 (s), 2853 (s), 1464 (m), 1389 (w), 1356 (w), 1254 (m), 1099 (m), 1057 (m), 1007 (m), 941 (w), 834 (s), 814 (m), 774 (s), 721 (w), 661 (w). All data in accordance with literature.^[6]

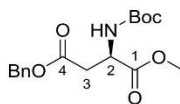
S-*tert*-butyl 4-bromo-3-oxo-butanethioate



To a solution of 2.883 g (20.0 mmol, 1.00 eq) Meldrum's acid in 30 mL dry CH₂Cl₂ at 0 °C were added 3.22 mL (40.0 mmol, 2.00 eq) pyridine and 1.91 mL (22.0 mmol, 1.10 eq) 2-bromoacetyl bromide over 10 min. The reaction mixture was warmed to room temperature and stirred at this temperature for 1 h. Then 2 M aqueous HBr was added. The aqueous phase was separated and extracted three times with CH₂Cl₂. The combined organic phases were dried over Na₂SO₄ and concentrated in vacuo. The residue was dissolved in 30 mL toluene at room temperature and 6.76 mL (60.0 mmol, 3.00 eq) *tert*-butylthiol were added over 10 min. The mixture was heated under reflux for 1 h and then concentrated in vacuo. The brownish residue was purified by column chromatography (silica, 95/5 cHex/EtOAc) to afford the title compound as a red oil: 3.828 g (15.121 mmol, 76%). In CDCl₃ the product exists as a tautomeric mixture of keto and enol forms in a ratio of 3.12:1.00; *R*_f = 0.66 (4:1 cHex/EtOAc); ¹H NMR (300 MHz, CDCl₃) δ 12.68 (s, 1H; OH^o), 5.56 (s, 1H; 2-H^o), 4.03 (s, 2H; 2-H^k), 3.81 (s, 2H; 4-H^o), 3.77 (s, 2H; 4-H^k), 1.49 (s, 9H; *t*Bu^o), 1.46 ppm (s, 9H; *t*Bu^k); ¹³C NMR (75 MHz, CDCl₃) δ 196.9, 194.0, 192.2, 167.5, 101.2, 59.3, 55.1, 49.5, 48.9, 40.0, 34.4, 30.1, 29.7, 28.8 ppm. All data in accordance with literature.^[7,8]

S-tert-butyl 4-(diethoxyphosphono)-3-oxobutanethioate (9)

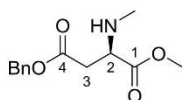
To a suspension of 0.503 g (21.862 mmol, 1.20 eq) sodium in 48 mL dry THF at room temperature were added 2.58 mL (20.040 mmol, 1.10 eq) diethyl phosphite. The mixture was heated under reflux for 5 h. In a separate flask, a suspension of 0.802 g (20.040 mmol, 1.10 eq) NaH in mineral oil in 32 mL dry THF at -40°C was treated with 4.612 g (18.218 mmol, 1.00 eq) of S-tert-butyl 4-bromo-3-oxo-butanethioate. After 30 min the solution of sodium diethyl phosphite was added to this mixture, kept at -20°C . The reaction mixture was slowly warmed to room temperature and then stirred at this temperature for 16 h. Saturated aqueous NH_4Cl was added and the resulting mixture was extracted three times with Et_2O . The combined organic phases were washed with H_2O and brine, dried over Na_2SO_4 and concentrated in vacuo. The crude yellow oil was purified by column chromatography (silica, 50/50 cHex/EtOAc) to afford the title compound as a red oil: 3.843 g (12.383 mmol, 68%). In CDCl_3 the product exists as a tautomeric mixture of keto and enol forms in a ratio of 3.50:1.00; $R_f = 0.28$ (1:3 cHex/EtOAc); ^1H NMR (300 MHz, CDCl_3): δ 12.93 (br s, 1H; OH^e), 5.44 (d, $J=3.2$ Hz, 1H; 2-H^e), 4.18-4.07 (m, 8H; CH_2CH_3), 3.77 (s, 2H; 2-H^k), 3.22 (d, $J=22.8$ Hz, 2H; 4-H^k), 2.69 (d, $J=22.1$ Hz, 2H; 4-H^e), 1.47 (s, 9H; tBu^e), 1.44 (s, 9H; tBu^k), 1.31 ppm (dt, $J=0.4, 7.1$ Hz, 12H; CH_3CH_2); ^{13}C NMR (75 MHz, CDCl_3): δ 194.2, 194.1, 192.3, 102.3, 102.2, 62.8, 62.7, 58.6, 49.3, 43.6, 41.9, 34.7, 32.9, 30.2, 29.7, 16.4, 16.3 ppm. All data in accordance with literature.^[7,8]

D-N-Boc-Asp(OBn)-OMe (15)

A solution of 1.617 g (5.00 mmol, 1.00 eq) D-Boc-Asp(OBn)-H (**14**) in 50 mL DMF at 0°C was treated with 0.829 g (6.00 mmol, 1.20 eq) K_2CO_3 and 0.47 mL (7.50 mmol, 1.50 eq) MeI. The mixture was warmed to room temperature and stirred at this temperature for 1 h. It was diluted with EtOAc and a 10 wt% aqueous solution of $\text{Na}_2\text{S}_2\text{O}_3$. The aqueous phase was separated and extracted three times with EtOAc. The combined organic phases were washed two times with H_2O and once with brine, dried over Na_2SO_4 , and concentrated in vacuo. The crude yellow oil was purified by column chromatography (silica, 75/25 cHex/EtOAc) to afford the title compound as a colourless solid: 1.523 g (4.517 mmol, 90%). $m_p = 63-64^{\circ}\text{C}$; $[\alpha]_D^{25} -31.6^{\circ}$ ($c = 1.00$, CHCl_3); $R_f = 0.72$ (1:1 cHex/EtOAc); ^1H NMR (500 MHz, CDCl_3) δ 7.40-7.30 (m, 5H; Ar-H), 5.48 (br d, $J=8.2$ Hz, 1H; NH), 5.15 (d, $J=12.2$ Hz, 1H; Ph-CH^aH^b), 5.11 (d, $J=12.2$ Hz, 1H; Ph-CH^aH^b), 4.59 (dt, $J=4.5, 8.2$ Hz, 1H; 2-H), 3.70 (s, 3H; OMe), 3.05 (dd, $J=4.5, 17.1$ Hz, 1H; 3-H^a), 2.87 (dd, $J=4.8, 17.1$ Hz, 1H; 3-H^b), 1.44 ppm (s, 9H; tBu); ^{13}C NMR (125 MHz, CDCl_3) δ 171.6, 170.9, 155.5, 135.5, 128.7, 128.6, 128.5, 80.3, 66.9, 52.8, 50.1, 37.1, 28.4 ppm; IR $\nu[\text{cm}^{-1}] = 3389$ (w), 2977 (w), 1714 (s), 1498 (m), 1456 (m), 1438 (m), 1391 (m),

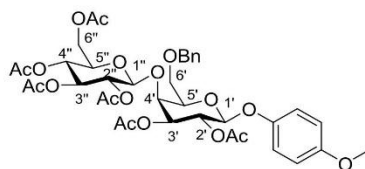
1346 (m), 1284 (m), 1214 (m), 1159 (s), 1048 (m), 1028 (m), 996 (m), 916 (w), 859 (w), 750 (m), 698 (m). All data in accordance with literature.^[9]

D-N-Me-Asp(OBn)-OMe (7)



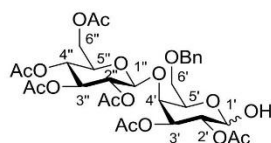
A solution of 0.337 g (1.00 mmol, 1.00 eq) D-N-Boc-Asp(OBn)-OMe (**15**) and 0.25 mL (4.00 mmol, 4.00 eq) MeI in 8 mL dry DMF was added dropwise to a suspension of 0.048 g (1.20 mmol, 1.20 eq) NaH in mineral oil in 4 mL dry DMF at 0 °C. The reaction was warmed to room temperature and stirred at this temperature for 1.5 h. Then the mixture was diluted with EtOAc and saturated aqueous NH₄Cl. The aqueous phase was separated and extracted three times with EtOAc. The combined organic phases were washed with brine, dried over Na₂SO₄, and concentrated in vacuo. The crude yellow oil was purified by column chromatography (silica, 85/15 cHex/EtOAc). The colourless oil thus obtained was dissolved in 8.7 mL CH₂Cl₂ and treated dropwise with 1.34 mL (17.444 mmol, 20.0 eq) TFA at 0 °C. The reaction mixture was warmed to room temperature, stirred at this temperature for 2 h, and then diluted with CH₂Cl₂ and a saturated aqueous NaHCO₃ solution. The aqueous phase was separated and extracted three times with CH₂Cl₂. The combined organic phases were washed with H₂O, dried over Na₂SO₄ and concentrated in vacuo. The colourless oily remainder was purified by column chromatography (silica, 65/35 cHex/EtOAc) to afford the title compound as a colourless oil: 0.172 g (0.684 mmol, 68%). $[\alpha]_D^{25} +5.5^\circ$ (c = 1.00, CHCl₃); R_f = 0.07 (2:1 cHex/EtOAc); ¹H NMR (500 MHz, CDCl₃) δ 7.39-7.30 (m, 5H; Ar-H), 5.16 (d, $J=12.3$ Hz, 1H; Ph-CH^aH^b), 5.11 (d, $J=12.3$ Hz, 1H; Ph-CH^aH^b), 3.70 (s, 3H; OMe), 3.59 (dd, $J=5.9, 7.0$ Hz, 1H; 2-H), 2.79 (dd, $J=5.9, 15.9$ Hz, 1H; 3-H^a), 2.70 (dd, $J=7.0, 15.9$ Hz, 1H; 3-H^b), 2.41 (s, 3H; NMe), 1.71 ppm (s, 1H; NH); ¹³C NMR (125 MHz, CDCl₃) δ 174.0, 170.8, 135.8, 128.7, 128.44, 128.42, 66.8, 59.4, 52.2, 37.7, 34.8 ppm; IR ν [cm⁻¹] = 3348 (w), 2952 (w), 2803 (w), 1732 (s), 1498 (w), 1456 (w), 1436 (w), 1384 (w), 1353 (w), 1261 (m), 1167 (m), 1121 (m), 1026 (w), 996 (w), 976 (w), 915 (w), 845 (w), 750 (m), 698 (m), 575 (w); HRMS (ESI⁺) m/z [M+H⁺] calcd for C₁₃H₁₈O₄N⁺ 252.12303, found 252.12234.

para-Methoxyphenyl-(2'',3'',4'',6''-tetra-O-acetyl- β -D-glucopyranosyl)-(1 \rightarrow 4)-2',3'-di-O-acetyl-6'-O-benzyl-1'-O- β -D-galactopyranoside (**24**)



A mixture of 0.799 g (1.735 mmol, 1.00 eq) acceptor **13**, 1.114 g (2.182 mmol, 1.25 eq) donor **12**, 35 mL dry CH₂Cl₂, and 1.50 g freshly activated molecular sieves (4 Å) was stirred at room temperature for 30 min. It was cooled to -78 °C and treated with 0.507 g (2.256 mmol, 1.30 eq) NIS and 15 µL (0.174 mmol, 0.10 eq) TfOH. The reaction mixture was warmed to 0 °C over 2 h and stirred for a further hour at this temperature before being stopped by adding 0.024 mL (0.174 mmol, 0.10 eq) NEt₃. The suspension was filtered through Celite® and washed with a saturated aqueous NaHCO₃ solution. The aqueous phase was separated and extracted three times with CH₂Cl₂. The combined organic phases were washed with a 10 wt% aqueous Na₂S₂O₃ solution and H₂O, dried over Na₂SO₄ and concentrated in vacuo. The yellow foam thus obtained was purified by column chromatography (silica, 50/50 cHex/EtOAc) to afford the title compound as a colourless solid: 1.119 g (1.415 mmol, 82%). *m*_p = 84-85 °C; [α]_D²⁵ -1.4 ° (c = 1.00, CHCl₃); *R*_f = 0.20 (1:1 cHex/EtOAc); ¹H NMR (500 MHz, CDCl₃) δ 7.36-7.25 (m, 5H; Ar-H), 7.01-6.96 (m, 2H; Ar-H), 6.80-6.75 (m, 2H; Ar-H), 5.32 (dd, *J*=7.9, 10.3 Hz, 1H; 2'-H), 5.25 (dd, *J*=9.6, 9.7 Hz, 1H; 3''-H), 5.09 (dd, *J*=9.6, 10.0 Hz, 1H; 4''-H), 5.04 (dd, *J*=8.0, 9.7 Hz, 1H; 2''-H), 5.00 (dd, *J*=3.1, 10.3 Hz, 1H; 3'-H), 4.88 (d, *J*=7.9 Hz, 1H; 1'-H), 4.59 (d, *J*=8.0 Hz, 1H; 1''-H), 4.55 (s, 2H; PhCH₂), 4.21-4.16 (m, 2H; 4'-H, 6''-H^a), 4.09 (dd, *J*=2.4, 12.1 Hz, 1H; 6''-H^b), 3.84-3.76 (m, 2H; 5'-H, 6'-H^a), 3.75 (s, 3H; OMe), 3.72 (dd, *J*=6.6, 10.1 Hz, 1H; 6'-H^b), 3.64 (ddd, *J*=2.4, 4.5, 10.0 Hz, 1H; 5''-H), 2.20 (s, 3H; CH₃-Ac), 2.15 (s, 3H; CH₃-Ac), 2.06 (s, 3H; CH₃-Ac), 2.05 (s, 3H; CH₃-Ac), 2.03 (s, 3H; CH₃-Ac), 2.03 ppm (s, 3H; CH₃-Ac); ¹³C NMR (125 MHz, CDCl₃) δ 170.4, 170.3, 170.2, 169.5, 169.4, 169.3, 155.4, 151.1, 138.2, 128.3, 127.6, 127.4, 118.5, 114.4, 101.2, 100.4, 74.2, 73.8, 73.4, 73.3, 72.6, 71.7, 71.3, 69.3, 69.2, 68.2, 61.6, 55.5, 20.8, 20.7, 20.62, 20.59, 20.52, 20.49 ppm; IR ν[cm⁻¹] = 1745 (s), 1507 (m), 1433 (w), 1367 (m), 1210 (s), 1168 (m), 1033 (s), 909 (m), 830 (w), 796 (w), 731 (m), 699 (m); HRMS (ESI⁺) *m/z* [M+Na⁺] calcd for C₃₈H₄₆O₁₈Na⁺ 813.25764, found 813.25635.

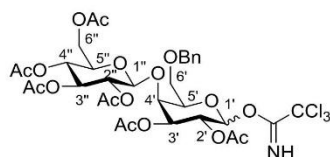
(2'',3'',4'',6''-tetra-*O*-Acetyl-β-D-glucopyranosyl)-(1→4)-2',3'-di-*O*-acetyl-6'-*O*-benzyl-D-galactopyranoside (25**)**



A solution of 0.395 g (0.50 mmol, 1.00 eq) disaccharide **24** in 3.75 mL acetone and 1.25 mL H₂O at 0 °C was treated with 1.369 g (2.498 mmol, 5.00 eq) CAN. The resulting mixture was warmed to room temperature, stirred for 30 min, concentrated in vacuo, and diluted with EtOAc and a saturated aqueous NaHCO₃ solution. The aqueous phase was separated and extracted three times with EtOAc. The combined organic phases were washed with an aqueous 10 wt% Na₂S₂O₃ solution and brine, dried over Na₂SO₄ and concentrated in vacuo. The remaining orange oil was purified by column chromatography (silica, 60/40 cHex/EtOAc) to afford the title compound as a pale yellow foam: 0.251 g

(0.367 mmol, 73%). $[\alpha]^{25}_{\text{D}} +29.0^\circ$ ($c = 1.0$, CHCl_3); $R_f = 0.40$ (1:2 cHex/EtOAc); $\alpha/\beta = 1.00/1.87$; ^1H NMR (500 MHz, CDCl_3) δ (β -product) 7.33-7.24 (m, 5H; Ar-H), 5.22-5.14 (m, 1H; 3''-H), 5.03-4.92 (m, 2H; 3'-H, 2''-H), 4.59 (dd, $J=6.4, 6.8$ Hz, 1H; 1'-H), 4.57-4.43 (m, 2H; PhCH_2), 4.43 (d, $J=8.0$ Hz, 1H; 1''-H), 4.33 (br d, $J=7.4$ Hz, 1H; OH), 4.30 (dd, $J=4.0, 7.6$ Hz, 1H; 5'-H), 4.18-4.08 (m, 2H; 6''-H), 4.11-4.07 (m, 1H; 4'-H), 3.73-3.69 (m, 1H; 4''-H), 3.67-3.61 (m, 2H; 6'-H), 3.62-3.53 (m, 1H; 5''-H), 2.12 (s, 3H; $\text{CH}_3\text{-Ac}$), 2.10 (s, 3H; $\text{CH}_3\text{-Ac}$), 2.04 (s, 3H; $\text{CH}_3\text{-Ac}$), 2.01 (s, 3H; $\text{CH}_3\text{-Ac}$), 2.01 (s, 3H; $\text{CH}_3\text{-Ac}$), 2.00 ppm (s, 3H; $\text{CH}_3\text{-Ac}$); ^{13}C NMR (125 MHz, CDCl_3) δ 138.0, 128.5, 127.8, 127.6, 101.3, 95.4, 74.5, 73.4, 73.3, 73.1, 72.5, 71.8, 71.3, 71.2, 69.1, 68.2, 61.7, 20.9, 20.7, 20.61, 20.58 ppm; IR $\nu[\text{cm}^{-1}] = 3441$ (w), 1741 (s), 1435 (w), 1368 (s), 1215 (s), 1158 (m), 1035 (s), 911 (m), 730 (s), 699 (m); HRMS (ESI^+) m/z $[\text{M}+\text{Na}^+]$ calcd for $\text{C}_{31}\text{H}_{40}\text{O}_{17}\text{Na}^+$ 707.21577, found 707.21484.

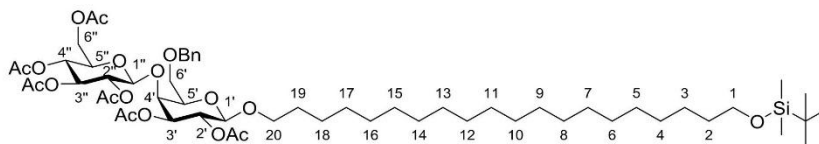
(2'',3'',4'',6''-tetra-*O*-Acetyl- β -D-glucopyranosyl)-(1 \rightarrow 4)-2',3'-di-*O*-acetyl-6'-*O*-benzyl-D-galactopyranosyl-trichloroacetimidate (10)



A solution of 0.497 g (0.726 mmol, 1.00 eq) disaccharide **25** in 14.5 mL dry CH_2Cl_2 at 0°C was treated with 0.44 mL (4.356 mmol, 6.00 eq) Cl_3CCN , and 0.171 g (1.234 mmol, 1.70 eq) of freshly dried K_2CO_3 . The reaction mixture was stirred for 2 h, warmed to room temperature, and stirred for 16 h. It was filtered through Celite® and concentrated in vacuo. The residual brown oil was purified by column chromatography (silica, 60/40 cHex/EtOAc) to afford the title compound as a separable mixture of α isomer as a colourless solid 0.286 g (0.345 mmol, 48%) and the β isomer as a colourless foam 0.174 g (0.210 mmol, 29%). *α -isomer*: $m_p = 167^\circ\text{C}$; $[\alpha]^{25}_{\text{D}} +4.8^\circ$ ($c = 1.00$, CHCl_3); $R_f = 0.60$ (1:2 cHex/EtOAc); ^1H NMR (500 MHz, CDCl_3) δ 8.61 (s, 1H; NH), 7.33-7.23 (m, 5H; Ar-H), 6.49 (d, $J=2.6$ Hz, 1H; 1'-H), 5.38-5.31 (m, 2H; 2'-H, 4'-H), 5.20 (dd, $J=9.6, 9.7$ Hz, 1H; 3''-H), 5.05 (dd, $J=9.6, 9.7$ Hz, 1H; 4''-H), 5.02 (dd, $J=8.0, 9.7$ Hz, 1H; 2''-H), 4.57-4.47 (m, 3H; PhCH_2 , 1''-H), 4.36-4.33 (m, 1H; 3'-H), 4.30 (dd, $J=5.7, 5.8$ Hz, 1H; 5''-H), 4.17 (dd, $J=4.7, 12.3$ Hz, 1H; 6'-H^a), 4.00 (dd, $J=2.1, 12.3$ Hz, 1H; 6'-H^b), 3.71 (dd, $J=5.7, 10.4$ Hz, 1H; 6''-H^a), 3.63-3.58 (m, 2H; 5'-H, 6''-H^b), 2.15 (s, 3H; $\text{CH}_3\text{-Ac}$), 2.11 (s, 3H; $\text{CH}_3\text{-Ac}$), 2.01 (s, 9H; $\text{CH}_3\text{-Ac}$), 2.00 ppm (s, 3H; $\text{CH}_3\text{-Ac}$); ^{13}C NMR (125 MHz, CDCl_3) δ 170.6, 170.5, 170.3, 169.8, 169.5, 161.1, 138.2, 128.5, 127.7, 127.5, 101.3, 94.1, 91.1, 74.4, 73.2, 72.7, 72.0, 71.9, 71.5, 70.4, 68.7, 68.3, 66.9, 61.8, 21.1, 20.80, 20.79, 20.71, 20.67 ppm; IR $\nu[\text{cm}^{-1}] = 1743$ (s), 1675 (w), 1432 (w), 1368 (m), 1214 (s), 1144 (m), 1031 (s), 970 (m), 941 (m), 904 (m), 837 (w), 796 (m), 731 (s), 699 (m); HRMS (ESI^+) m/z $[\text{M}+\text{Na}^+]$ calcd for $\text{C}_{33}\text{H}_{40}\text{Cl}_3\text{NO}_{17}\text{Na}^+$ 850.12540, found 850.12445; *β -isomer*: $[\alpha]^{25}_{\text{D}} +3.3^\circ$ ($c = 1.00$, CHCl_3); R_f (1:2 cHex/EtOAc); ^1H NMR (500 MHz, CDCl_3) δ 8.68 (s, 1H; NH), 7.36-7.25 (m, 5H; Ar-H), 5.80 (d, $J=8.2$ Hz, 1H; 1'-H), 5.36 (dd, $J=8.2, 10.3$ Hz, 1H; 2'-H), 5.23 (dd, $J=9.5, 9.6$ Hz, 1H; 4''-H), 5.11-5.01 (m, 3H; 2''-H, 3''-H, 3'-H), 4.60 (d, $J=8.0$

Hz, 1H; 1''-H), 4.53 (s, 2H; PhCH₂), 4.24 (br d, $J=2.9$ Hz, 1H; 4'-H), 4.18 (dd, $J=4.4$, 12.3 Hz, 1H; 6'-H^a), 4.02 (dd, $J=2.3$, 12.3 Hz, 1H; 6'-H^b), 3.90 (dd, $J=5.7$, 5.9 Hz, 1H; 5''-H), 3.78 (dd, $J=5.7$, 10.2 Hz, 1H; 6''-H^a), 3.67 (dd, $J=5.9$, 10.2 Hz, 1H; 6''-H^b), 3.61 (ddd, $J=2.3$, 4.4, 9.9 Hz, 1H; 5'-H), 2.18 (s, 3H; CH₃-Ac), 2.14 (s, 3H; CH₃-Ac), 2.05 (s, 3H; CH₃-Ac), 2.03 (2s, 6H; CH₃-Ac), 2.00 ppm (s, 3H; CH₃-Ac); ¹³C NMR (125 MHz, CDCl₃) δ 170.6, 170.5, 170.2, 169.7, 169.5, 169.1, 161.3, 138.2, 128.5, 127.8, 127.7, 101.3, 96.1, 90.6, 74.8, 73.9, 73.35, 73.25, 72.8, 72.0, 71.5, 68.5, 68.4, 68.3, 61.7, 60.5, 20.9, 20.78, 20.77, 20.75, 20.70, 20.6 ppm; IR ν [cm⁻¹] = 1745 (s), 1678 (w), 1452 (w), 1432 (w), 1368 (m), 1289 (w), 1212 (s), 1168 (m), 1084 (m), 1032 (s), 951 (w), 909 (m), 837 (m), 796 (m), 732 (m), 699 (m), 647 (m), 599 (m).

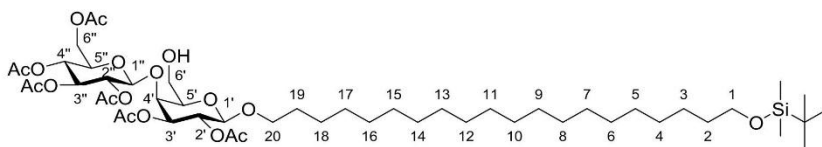
(1-(*tert*-Butyl-dimethyl-silyloxy)-eicosyl)-(2'',3'',4'',6''-tetra-*O*-acetyl- β -D-glucopyranosyl)-(1 \rightarrow 4)-2',3'-di-*O*-acetyl-6'-*O*-benzyl-1'-*O*- β -D-galactopyranoside (27)



A mixture of 0.335 g (0.404 mmol, 1.00 eq) donor **10**, 0.260 g (0.606 mmol, 1.50 eq) acceptor **11**, 0.60 g freshly activated molecular sieves (4 Å), and 13.5 mL dry CH₂Cl₂ was stirred at room temperature for 30 min. It was cooled to -40 °C and 7.3 μ L (0.0404 mmol, 0.10 eq) TMSOTf were added. The mixture was allowed to slowly warm to room temperature over 16 h, filtered through Celite®, and washed with a saturated aqueous NaHCO₃ solution. The aqueous phase was separated and extracted three times with CH₂Cl₂. The combined organic phases were washed with brine, dried over Na₂SO₄, and concentrated in vacuo. The remaining pale yellow waxy oil was purified by column chromatography (silica, 75/25 cHex/EtOAc) to afford 0.264 g (0.241 mmol, 60%) of the title compound as a colourless solid, aside of 0.093 g (0.088 mmol, 22%) of the 2''-deacetylated derivate. The latter was dissolved in 0.35 mL pyridine at room temperature and treated with 17 μ L (0.176 mmol, 2.00 eq) Ac₂O. The resulting mixture was stirred at this temperature for 16 h, diluted with EtOAc and a saturated aqueous NaHCO₃ solution, and the aqueous phase was separated and extracted three times with EtOAc. The combined organic phases were dried over Na₂SO₄ and concentrated in vacuo. The residue was purified by column chromatography (silica, 70/30 cHex/EtOAc) to afford 0.070 g (0.064 mmol, 73%) of the title compound as a colourless solid. $[\alpha]_D^{25} -4.8^\circ$ (c = 1.00, CHCl₃); R_f = 0.57 (1:1 cHex/EtOAc); ¹H NMR (500 MHz, CDCl₃) δ 7.34-7.24 (m, 5H; Ar-H), 5.20 (dd, $J=9.6$, 9.7 Hz, 1H; 3''-H), 5.06 (dd, $J=8.1$, 10.2 Hz, 1H; 2''-H), 5.04 (dd, $J=9.7$, 9.9 Hz, 1H; 4''-H), 4.98 (dd, $J=8.0$, 9.6 Hz, 1H; 2''-H), 4.91 (dd, $J=3.0$, 10.2 Hz, 1H; 3'-H), 4.54 (d, $J=8.0$ Hz, 1H; 1''-H), 4.53 (s, 2H; PhCH₂), 4.39 (d, $J=8.1$ Hz, 1H; 1'-H), 4.15 (dd, $J=4.5$, 12.4 Hz, 1H; 6''-H^a), 4.11 (d, $J=3.0$ Hz, 1H; 4'-H), 4.02 (dd, $J=2.2$, 12.4 Hz, 1H; 6''-H^b), 3.83 (dt, $J=6.3$, 9.6 Hz, 1H; 20-H^a), 3.73 (dd, $J=4.6$, 9.1 Hz, 1H; 6'-H^a), 3.69-3.62 (m, 2H; 1-H^a, 5'-H), 3.60-3.56 (m, 3H; 1-H^b, 5''-H, 6'-H^b), 3.43 (dt, $J=6.9$, 9.6 Hz, 1H; 20-H^b), 2.15 (s, 3H; CH₃-Ac), 2.10 (s, 3H; CH₃-Ac), 2.01 (s, 6H; CH₃-Ac), 2.00 (s, 6H; CH₃-Ac), 1.57-1.46 (m,

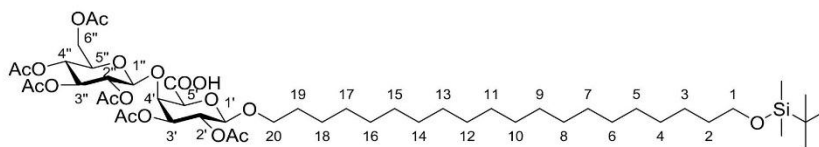
4H; 2-H, 19-H), 1.32-1.21 (m, 32H; 3-H to 18-H), 0.88 (s, 9H; *t*Bu), 0.04 ppm (s, 6H; CH₃-Si); ¹³C NMR (125 MHz, CDCl₃) δ 170.6, 170.5, 170.4, 169.6, 169.5, 169.4, 138.3, 128.5, 127.8, 127.6, 101.4, 100.9, 74.5, 73.6, 73.54, 73.47, 72.8, 71.9, 71.4, 69.7, 69.5, 69.3, 68.3, 63.5, 61.8, 33.0, 29.82, 29.79, 29.76, 29.7, 29.6, 29.52, 29.50, 26.1, 26.0, 25.9, 20.94, 20.86, 20.77, 20.7, 20.6, 18.5, -5.1 ppm; IR ν[cm⁻¹] = 3554 (br w), 2925 (m), 2854 (m), 1749 (s), 1464 (w), 1434 (w), 1368 (m), 1216 (s), 1170 (m), 1037 (s), 907 (w), 835 (m), 774 (m), 734 (m); HRMS (ESI⁺) *m/z* [M+Na⁺] calcd for C₅₇H₉₄O₁₈SiNa⁺ 1117.61016, found 1117.60981.

(1-(*tert*-Butyl-dimethyl-silyloxy)-eicosyl)-(2'',3'',4'',6''-tetra-*O*-acetyl-β-D-glucopyranosyl)-(1→4)-2',3'-di-*O*-acetyl-1'-*O*-β-D-galactopyranoside (28)



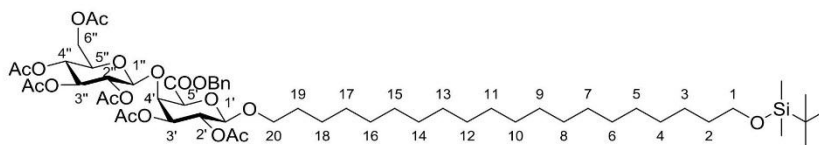
To a solution of 0.422 g (0.386 mmol, 1.00 eq) **27** in 10 mL dry EtOAc at room temperature was added 0.084 g 5% palladium on charcoal. The suspension was saturated with H₂ and stirred under 1 bar of H₂ gas for 3.5 h. The mixture was filtered through Celite® and concentrated in vacuo to afford 0.383 g (0.381 mmol, 99%) of the title compound as a colourless foam. The product was used without further purification. [α]_D²⁵ +1.9 ° (c = 1.00, CHCl₃); R_f = 0.33 (1:1 cHex/EtOAc); ¹H NMR (500 MHz, CDCl₃) δ 5.22 (dd, *J*=9.6, 9.7 Hz, 1H; 3''-H), 5.06-5.00 (m, 2H; 2'-H, 2''-H), 4.96 (dd, *J*=9.6, 9.8 Hz, 1H; 4''-H), 4.92 (dd, *J*=3.0, 10.3 Hz, 1H; 3'-H), 4.52 (d, *J*=8.1 Hz, 1H; 1''-H), 4.39 (d, *J*=7.8 Hz, 1H; 1'-H), 4.35 (dd, *J*=1.9, 12.1 Hz, 1H; 6''-H^a), 4.19 (d, *J*=3.0 Hz, 1H; 4'-H), 3.94 (dd, *J*=7.2, 12.2 Hz, 1H; 6''-H^b), 3.84-3.77 (m, 2H; 6'-H^a, 20-H^a), 3.71 (ddd, *J*=1.9, 7.1, 9.8 Hz, 1H; 5''-H), 3.66-3.60 (m, 1H; 6'-H^b), 3.60-3.54 (m, 3H; 5'-H, 1-H), 3.41 (dt, *J*=6.9, 9.5 Hz, 1H; 20-H^b), 2.91 (dd, *J*=7.0, 7.2 Hz, 1H; OH), 2.15 (s, 3H; CH₃-Ac), 2.11 (s, 3H; CH₃-Ac), 2.08 (s, 3H; CH₃-Ac), 2.03 (s, 3H; CH₃-Ac), 2.01 (s, 3H; CH₃-Ac), 2.00 (s, 3H; CH₃-Ac), 1.55-1.45 (m, 4H; 2-H, 19-H), 1.33-1.19 (m, 32H; 3-H to 18-H), 0.88 (s, 9H; *t*Bu-Si), 0.03 ppm (s, 6H; CH₃-Si); ¹³C NMR (125 MHz, CDCl₃) δ 170.7, 170.5, 170.4, 169.52, 169.49, 169.42, 102.0, 100.9, 74.2, 73.41, 73.38, 72.4, 72.0, 71.0, 69.8, 69.5, 68.7, 63.5, 62.3, 59.3, 33.0, 29.82, 29.76, 29.74, 29.6, 29.51, 29.48, 26.1, 25.94, 25.91, 20.94, 20.85, 20.74, 20.71, 20.69, 20.58, 18.5, -5.1 ppm; IR ν[cm⁻¹] = 3554 (br w), 2925 (m), 2854 (m), 1749 (s), 1464 (w), 1434 (w), 1368 (m), 1216 (s), 1170 (m), 1037 (s), 907 (w), 835 (m), 774 (m), 734 (m); HRMS (ESI⁺) *m/z* [M+Na⁺] calcd for C₅₀H₈₈O₁₈SiNa⁺ 1027.56321, found 1027.56105.

(1-(*tert*-Butyl-dimethyl-silyloxy)-eicosyl)-(2'',3'',4'',6''-tetra-*O*-acetyl- β -D-glucopyranosyl)-(1 \rightarrow 4)-2',3'-di-*O*-acetyl-1'-*O*- β -D-galactopyranosiduronic acid (29**)**



A mixture of 0.383 g (0.381 mmol, 1.00 eq) **28**, 5.1 mL CH₂Cl₂, and 2.5 mL H₂O was treated with 0.012 g (0.076 mmol, 0.20 eq) TEMPO and 0.270 g (0.838 mmol, 2.20 eq) BAIB at room temperature. After 1 h, another 0.012 g (0.076 mmol, 0.20 eq) of TEMPO were added and stirring continued for 1 h. The reaction mixture was diluted with EtOAc and a 10 wt% aqueous Na₂S₂O₃ solution, the aqueous phase was separated and extracted three times with EtOAc, and the combined organic phases were washed with brine, dried over Na₂SO₄ and concentrated in vacuo. The crude orange oil was purified by column chromatography (silica, 50/50 cHex/EtOAc \rightarrow 90/10 CH₂Cl₂/MeOH) to afford 0.377 g (0.370 mmol, 97%) of the title compound as a colourless foam. $[\alpha]_D^{25} +1.8^\circ$ (c = 1.00, CHCl₃); $R_f = 0.16$ (1:2 cHex/EtOAc + HCOOH); ¹H NMR (500 MHz, CDCl₃ + F₃CCOOH) δ 5.26 (dd, $J=9.5, 9.6$ Hz, 1H; 3'-H), 5.12 (dd, $J=9.5, 9.8$ Hz, 1H; 4''-H), 5.09 (dd, $J=7.8, 10.2$ Hz, 1H; 2''-H), 5.03-4.98 (m, 2H; 2'-H, 4'-H), 4.68 (dd, $J=1.9, 12.5$ Hz, 1H; 6''-H^a), 4.52 (d, $J=8.0$ Hz, 1H; 1'-H), 4.50 (d, $J=7.8$ Hz, 1H; 1''-H), 4.47-4.44 (m, 1H; 5'-H), 4.25 (s, 1H; 3''-H), 3.93-3.87 (m, 2H; 20-H^a, 6''-H^b), 3.75 (t, $J=7.1$ Hz, 2H; 1-H), 3.66 (dt, $J=2.8, 9.8$ Hz, 1H; 5''-H), 3.47 (dt, $J=6.9, 9.6$ Hz, 1H; 20-H^b), 2.18 (s, 3H; CH₃-Ac), 2.16 (s, 3H; CH₃-Ac), 2.15 (s, 3H; CH₃-Ac), 2.07 (s, 6H; CH₃-Ac), 2.06 (s, 3H; CH₃-Ac), 1.61-1.49 (m, 4H; 2-H, 19-H), 1.34-1.18 (m, 32H; 3-H to 18-H), 0.92 (s, 9H; *t*Bu), 0.14 ppm (s, 6H; CH₃-Si); ¹³C NMR (125 MHz, CDCl₃ + F₃CCOOH) δ 174.4, 172.2, 171.39, 171.37, 171.0, 170.7, 169.6, 101.1, 100.5, 75.5, 73.0, 72.93, 72.90, 71.7, 71.5, 70.6, 69.1, 68.3, 64.8, 61.0, 32.2, 29.9, 29.82, 29.79, 29.78, 29.74, 29.71, 29.69, 29.5, 29.3, 26.0, 25.8, 25.6, 21.1, 20.83, 20.80, 20.76, 20.74, 20.6, 18.7, -5.2 ppm; IR ν [cm⁻¹] = 2925 (m), 2854 (m), 1748 (s), 1464 (w), 1433 (w), 1367 (m), 1218 (s), 1169 (m), 1038 (s), 980 (w), 926 (w), 911 (w), 835 (m), 696 (w), 659 (w), 599 (m), 555 (w); HRMS (ESI⁺) m/z [M+Na⁺] calcd for C₅₀H₈₆O₁₉SiNa⁺ 1041.54248 found 1041.54028.

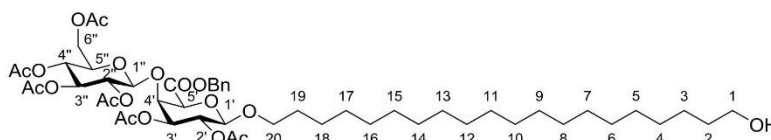
Benzyl-((1-(*tert*-butyl-dimethyl-silyloxy)-eicosyl)-(2'',3'',4'',6''-tetra-*O*-acetyl- β -D-glucopyranosyl)-(1 \rightarrow 4)-2',3'-di-*O*-acetyl-1'-*O*- β -D-galactopyranoside) uronate (30**)**



A solution of 0.138 g (0.135 mmol, 1.00 eq) **29** in 3 mL dry DMF at 0 °C was treated with 0.023 g (0.162 mmol, 1.20 eq) K₂CO₃ and 20.9 μ L (0.176 mmol, 1.30 eq) benzyl bromide. The reaction mixture

was warmed to room temperature, stirred for 5 h, and then diluted with EtOAc and a saturated aqueous NH_4Cl solution. The aqueous phase was separated and extracted three times with EtOAc. The combined organic phases were washed with brine, dried over Na_2SO_4 and concentrated in vacuo. The remaining colourless oil was purified by column chromatography (silica, 70/30 cHex/EtOAc) to afford 0.139 g (0.125 mmol, 93%) of the title compound as a colourless foam. $[\alpha]_D^{25} -6.4^\circ$ ($c = 1.00$, CHCl_3); $R_f = 0.48$ (1:1 cHex/EtOAc); ^1H NMR (500 MHz, CDCl_3) δ 7.40-7.29 (m, 5H; Ar-H), 5.33 (d, $J=12.3$ Hz, 1H; PhCH^aH^b), 5.21 (dd, $J=9.5, 9.6$ Hz, 1H; $3''\text{-H}$), 5.17 (dd, $J=8.0, 10.3$ Hz, 1H; $2'\text{-H}$), 5.08 (d, $J=12.5$ Hz, 1H; PhCH^aH^b), 4.97 (dd, $J=3.0, 10.3$ Hz, 1H; $3'\text{-H}$), 4.94 (dd, $J=9.6, 9.8$ Hz, 1H; $4''\text{-H}$), 4.89 (dd, $J=8.0, 9.5$ Hz, 1H; $2''\text{-H}$), 4.59 (d, $J=8.0$ Hz, 1H; $1''\text{-H}$), 4.49 (dd, $J=1.0, 3.0$ Hz, 1H; $4'\text{-H}$), 4.42 (d, $J=8.0$ Hz, 1H; $1'\text{-H}$), 4.24 (dd, $J=4.7, 12.3$ Hz, 1H; $6''\text{-H}^a$), 4.19-4.17 (m, 1H; $5'\text{-H}$), 3.94-3.87 (m, 2H; $6''\text{-H}^b, 20\text{-H}^a$), 3.62-3.57 (m, 3H; 1-H, 1-H, $5''\text{-H}$), 3.44 (dt, $J=6.9, 9.5$ Hz, 1H; 20-H^b), 2.14 (s, 3H; $\text{CH}_3\text{-Ac}$), 2.13 (s, 3H; $\text{CH}_3\text{-Ac}$), 2.03 (s, 3H; $\text{CH}_3\text{-Ac}$), 2.01 (s, 3H; $\text{CH}_3\text{-Ac}$), 2.00 (s, 3H; $\text{CH}_3\text{-Ac}$), 1.99 (s, 3H; $\text{CH}_3\text{-Ac}$), 1.60-1.45 (m, 4H; 2-H, 19-H), 1.33-1.18 (m, 32H; 3-H to 18-H), 0.89 (s, 9H; $t\text{Bu}$), 0.04 ppm (s, 6H; $\text{CH}_3\text{-Si}$); ^{13}C NMR (125 MHz, CDCl_3) δ 170.8, 170.6, 170.4, 169.53, 169.48, 169.3, 166.2, 135.6, 128.7, 128.5, 128.1, 101.2, 100.8, 75.2, 73.9, 73.1, 72.8, 72.0, 71.8, 70.0, 68.9, 68.2, 67.0, 63.5, 61.8, 33.0, 29.9, 29.84, 29.80, 29.79, 29.6, 29.5, 29.4, 26.1, 26.0, 20.9, 20.85, 20.82, 20.78, 20.72, 20.68, 18.5, -5.1 ppm; IR $\nu[\text{cm}^{-1}] = 2926$ (s), 2854 (s), 1751 (s), 1464 (w), 1433 (w), 1368 (m), 1222 (s), 1167 (w), 1090 (m), 1045 (m), 836 (w); HRMS (ESI $^+$) m/z $[\text{M}+\text{Na}^+]$ calcd for $\text{C}_{57}\text{H}_{92}\text{O}_{19}\text{SiNa}^+$ 1131.58943, found 1131.58541.

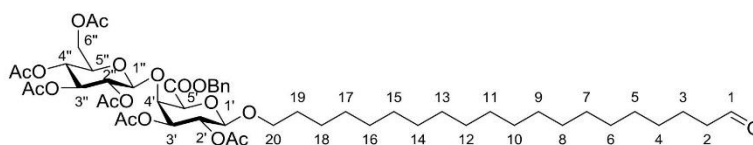
Benzyl-(1-hydroxy-eicosyl-(2'',3'',4'',6''-tetra-O-acetyl- β -D-glucopyranosyl)-(1 \rightarrow 4)-2',3'-di-O-acetyl-1'-O- β -D-galactopyranoside) uronate (31)



A solution of 0.441 g (0.398 mmol, 1.00 eq) **30** in 2 mL THF, 2 mL H_2O and 6 mL AcOH was stirred at room temperature for 2.5 h. EtOAc and a saturated aqueous NaHCO_3 solution were added and the aqueous phase was separated and extracted three times with EtOAc. The combined organic phases were washed with brine, dried over Na_2SO_4 and concentrated in vacuo. The colourless oily residue was purified by column chromatography (silica, 50/50 cHex/EtOAc) to afford 0.365 g (0.367 mmol, 92%) of the title compound as a colourless syrup. $[\alpha]_D^{25} -9.3^\circ$ ($c = 1.0$, CHCl_3); $R_f = 0.15$ (1:1 cHex/EtOAc); ^1H NMR (500 MHz, CDCl_3) δ 7.40-7.30 (m, 5H; Ar-H), 5.32 (d, $J=12.6$ Hz, 1H; PhCH^aH^b), 5.22-5.14 (m, 2H; $2'\text{-H}$, $3''\text{-H}$), 5.07 (d, $J=12.6$ Hz, 1H; PhCH^aH^b), 4.97 (dd, $J=9.7$ Hz, 1H; $3'\text{-H}$), 4.94 (dd, $J=10.0$ Hz, 1H; $4''\text{-H}$), 4.89 (dd, $J=8.1, 9.6$ Hz, 1H; $2''\text{-H}$), 4.59 (d, $J=8.1$ Hz, 1H; $1''\text{-H}$), 4.51-4.47 (m, 1H; $4'\text{-H}$), 4.41 (d, $J=7.9$ Hz, 1H; $1'\text{-H}$), 4.24 (dd, $J=4.8, 12.3$ Hz, 1H; $6''\text{-H}^a$), 4.19-4.16 (m, 1H; $5'\text{-H}$), 3.94-3.86 (m, 2H; $20\text{-H}^a, 6''\text{-H}^b$), 3.63 (t, $J=6.7$ Hz, 2H; 1-H), 3.58 (ddd, $J=2.2, 4.8, 10.0$ Hz, 1H; $5''\text{-H}$), 3.47-3.41 (m, 1H; 20-H^b), 2.13 (s, 3H; $\text{CH}_3\text{-Ac}$), 2.12 (s, 3H; $\text{CH}_3\text{-Ac}$), 2.01 (s, 3H; $\text{CH}_3\text{-Ac}$), 1.99 (s, 3H; $\text{CH}_3\text{-Ac}$), 1.60-1.45 (m, 4H; 2-H, 19-H), 1.33-1.18 (m, 32H; 3-H to 18-H), 0.89 (s, 9H; $t\text{Bu}$), 0.04 ppm (s, 6H; $\text{CH}_3\text{-Si}$); ^{13}C NMR (125 MHz, CDCl_3) δ 170.8, 170.6, 170.4, 169.53, 169.48, 169.3, 166.2, 135.6, 128.7, 128.5, 128.1, 101.2, 100.8, 75.2, 73.9, 73.1, 72.8, 72.0, 71.8, 70.0, 68.9, 68.2, 67.0, 63.5, 61.8, 33.0, 29.9, 29.84, 29.80, 29.79, 29.6, 29.5, 29.4, 26.1, 26.0, 20.9, 20.85, 20.82, 20.78, 20.72, 20.68, 18.5, -5.1 ppm; IR $\nu[\text{cm}^{-1}] = 2926$ (s), 2854 (s), 1751 (s), 1464 (w), 1433 (w), 1368 (m), 1222 (s), 1167 (w), 1090 (m), 1045 (m), 836 (w); HRMS (ESI $^+$) m/z $[\text{M}+\text{Na}^+]$ calcd for $\text{C}_{57}\text{H}_{92}\text{O}_{19}\text{SiNa}^+$ 1131.58943, found 1131.58541.

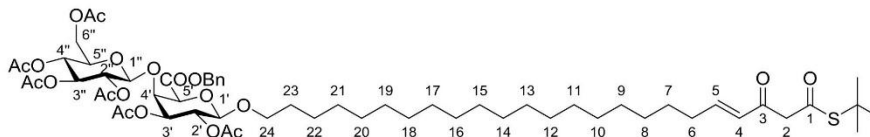
Ac), 2.00 (s, 6H; CH₃-Ac), 1.99 (s, 3H; CH₃-Ac), 1.60-1.48 (m, 4H; 2-H, 19-H), 1.35-1.19 ppm (m, 32H; 3-H to 18-H); ¹³C NMR (125 MHz, CDCl₃) δ 170.8, 170.6, 170.4, 169.51, 169.46, 169.3, 166.2, 135.6, 128.7, 128.5, 128.1, 101.2, 100.7, 75.2, 73.8, 73.0, 72.8, 71.9, 71.7, 69.9, 68.8, 68.2, 67.0, 63.2, 61.8, 32.9, 29.8, 29.7, 29.6, 29.5, 29.4, 25.9, 25.8, 20.9, 20.82, 20.79, 20.75, 20.69, 20.66 ppm; IR ν[cm⁻¹] = 3507 (br w), 2924 (m), 2853 (m), 1747 (s), 1433 (w), 1367 (m), 1216 (s); 1168 (m), 1121 (m), 1037 (s), 911 (m), 813 (w), 774 (w), 732 (m), 698 (w), 647 (w), 599 (w); HRMS (ESI⁺) m/z [M+Na⁺] calcd for C₅₁H₇₆O₁₉Na⁺ 1017.50295 found 1017.50029.

Benzyl-(1-oxo-eicosyl-(2'',3'',4'',6''-tetra-O-acetyl-β-D-glucopyranosyl)-(1→4)-2',3'-di-O-acetyl-1'-O-β-D-galactopyranoside) uronate (8)



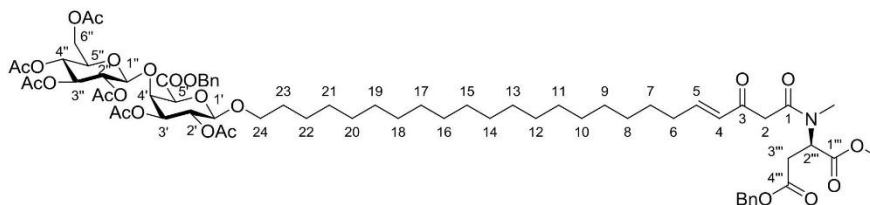
A solution of 0.365 g (0.367 mmol, 1.00 eq) alcohol **31** in 8 mL dry CH₂Cl₂ at room temperature was treated with 0.233 g (0.550 mmol, 1.50 eq) DMP, stirred for 2 h at this temperature, and then treated with a 10 wt% aqueous solution of Na₂S₂O₃ and a saturated aqueous NaHCO₃ solution. The aqueous phase was separated and extracted three times with EtOAc. The combined organic phases were washed with brine, dried over Na₂SO₄ and concentrated in vacuo. The remaining colourless oil was purified by column chromatography (silica, 50/50 cHex/EtOAc) to afford 0.242 g (0.244 mmol, 79%, based on recovered starting material) of the title compound as a colourless syrup. [α]_D²⁵ -10.9 ° (c = 1.00, CHCl₃); R_f = 0.38 (1:1 cHex/EtOAc); ¹H NMR (500 MHz, CDCl₃) δ 9.76 (t, J=1.9 Hz, 1H; 1-H), 7.42-7.28 (m, 5H; Ar-H), 5.33 (d, J=12.5 Hz, 1H; PhCH^aH^b), 5.20 (dd, J=9.5, 9.6 Hz, 1H; 3''-H), 5.16 (dd, J=7.9, 10.3 Hz, 1H; 2''-H), 5.08 (d, J=12.5 Hz, 1H; PhCH^aH^b), 4.97 (dd, J=3.0, 10.3 Hz, 1H; 3'-H), 4.95 (dd, J=9.5, 9.8 Hz, 1H; 4''-H), 4.90 (dd, J=8.0, 9.6 Hz, 1H; 2''-H), 4.59 (dd, J=8.0 Hz, 1H; 1''-H), 4.49 (dd, J=0.9, 3.0 Hz, 1H; 4'-H), 4.42 (d, J=7.9 Hz, 1H; 1'-H), 4.24 (dd, J=4.8, 12.4 Hz, 1H; 6''-H^a), 4.18 (d, J=0.9 Hz, 1H; 5'-H), 3.94-3.87 (m, 2H; 6''-H^b, 20-H^a), 3.59 (ddd, J=2.3, 4.8, 9.8 Hz, 1H; 5''-H), 3.44 (dt, J=6.8, 9.5 Hz, 1H; 20-H^b), 2.42 (dt, J=1.9, 7.4 Hz, 2H; 2-H), 2.14 (s, 3H; CH₃-Ac), 2.13 (s, 3H; CH₃-Ac), 2.02 (s, 3H; CH₃-Ac), 2.01 (s, 3H; CH₃-Ac), 2.00 (s, 6H; CH₃-Ac), 1.65-1.59 (m, 2H; 3-H), 1.59-1.51 (m, 2H; 19-H), 1.38-1.16 ppm (m, 30H; 4-H to 18-H); ¹³C NMR (125 MHz, CDCl₃) δ 203.2, 170.8, 170.6, 170.4, 169.53, 169.48, 169.3, 166.2, 135.6, 128.7, 128.5, 128.1, 101.2, 100.8, 75.2, 73.9, 73.1, 73.0, 71.9, 71.7, 70.0, 69.9, 68.2, 67.0, 61.8, 44.1, 29.84, 29.82, 29.7, 29.6, 29.5, 29.4, 29.3, 26.0, 22.2, 20.9, 20.85, 20.81, 20.78, 20.71, 20.68 ppm; IR ν[cm⁻¹] = 2924 (m), 2854 (m), 1750 (s), 1459 (w), 1434 (w), 1368 (m), 1221 (s), 1168 (w), 1123 (w), 1085 (m), 1042 (s), 919 (w), 773 (w), 735 (w), 698 (w), 601 (w), 576 (w), 555 (w); HRMS (ESI⁺) m/z [M+Na⁺] calcd for C₅₁H₇₆O₁₉Na⁺ 1015.48730, found 1015.48548.

(4E)-Benzyl-(3-oxo-1-(S-tert-butylthiocarbonyl-eicosa-4-enyl)-(2'',3'',4'',6'')-tetra-O-acetyl-β-D-glucopyranosyl)-(1→4)-2',3'-di-O-acetyl-1'-O-β-D-galactopyranoside) uronate (6)



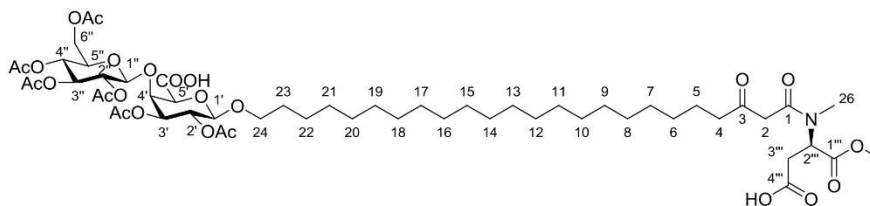
To a suspension of 0.029 g (0.731 mmol, 3.00 eq) NaH on mineral oil in 1.5 mL dry THF at 0 °C was dropwise added a solution of 0.113 g (0.366 mmol, 1.50 eq) phosphonate **9** in 2.5 mL THF. The reaction mixture was stirred for 1 h at this temperature. Then 0.242 g (0.244 mmol, 1.00 eq) aldehyde **8** dissolved in 7 mL dry THF were added at 0 °C. The reaction was slowly warmed to room temperature over 16 h. Then a saturated aqueous NH₄Cl solution was added. The aqueous phase was separated and extracted three times with Et₂O. The combined organic phases were washed with brine, dried over Na₂SO₄ and concentrated in vacuo. The remaining yellow oil was purified by column chromatography (silica, 70/30 cHex/EtOAc) to afford 0.127 g (0.111 mmol, 45%) of the title compound as a pale yellow syrup. In CDCl₃ the product exists as a tautomeric mixture of keto and enol forms in a ratio of 1.00:1.00; [α]_D²⁵ -1.1 ° (c = 1.00, CHCl₃); R_f = 0.56 (1:1 cHex/EtOAc); ¹H NMR (500 MHz, CDCl₃) δ 12.61 (d, J=1.4 Hz, 1H; OH), 7.42-7.31 (m, 5H; Ar-H), 6.91 (dt, J=7.0, 15.9 Hz, 1H; 5-H keto), 6.70 (dt, J=7.2, 15.4 Hz, 1H; 5-H enol), 6.15 (dt, J=1.3, 15.9 Hz, 1H; 4-H keto), 5.67 (ddd, J=1.2, 2.7, 15.5 Hz, 1H; 4-H enol), 5.33 (d, J=12.5 Hz, 1H; PhCH^aH^b), 5.31 (s, 2H; 2-H keto), 5.20 (dd, J=9.5, 9.6 Hz, 1H; 3''-H), 5.17 (dd, J=8.0, 10.3 Hz, 1H; 2''-H), 5.08 (d, J=12.5 Hz, 1H; PhCH^aH^b), 4.97 (dd, J=3.0, 10.3 Hz, 1H; 3'-H), 4.95 (dd, J=9.6, 9.9 Hz, 1H; 4''-H), 4.90 (dd, J=8.0, 9.5 Hz, 1H; 2''-H), 4.59 (d, J=8.0 Hz, 1H; 1''-H), 4.50 (dd, J=0.9, 3.0 Hz, 1H; 4'-H), 4.42 (d, J=8.0 Hz, 1H; 1'-H), 4.23 (dd, J=4.8, 12.4 Hz, 1H; 6''-H^a), 4.18 (s, 1H; 5'-H), 3.94-3.87 (m, 2H; 6''-H^b, 24-H^a), 3.70 (s, 1H; 2-H enol), 3.59 (ddd, J=2.2, 4.8, 9.9 Hz, 1H; 5''-H), 3.44 (dt, J=6.9, 9.5 Hz, 1H; 24-H^b), 2.24 (ddd, J=1.2, 7.2, 14.5 Hz, 2H, 6-H keto), 2.17 (ddd, J=1.2, 7.2, 14.6 Hz, 2H; 6-H enol), 2.14 (s, 3H; CH₃-Ac), 2.13 (s, 3H; CH₃-Ac), 2.02 (s, 3H; CH₃-Ac), 2.01 (s, 3H; CH₃-Ac), 2.00 (s, 6H; CH₃-Ac), 1.60-1.53 (m, 2H; 23-H), 1.51 (s, 9H; tBu keto), 1.47 (s, 9H; tBu enol), 1.48-1.40 (m, 2H; 7-H), 1.35-1.20 ppm (m, 30H; 8-H to 22-H); ¹³C NMR (125 MHz, CDCl₃) δ 196.5, 170.8, 170.6, 170.4, 169.53, 169.48, 169.3, 166.2, 151.0, 143.0, 135.6, 129.7, 128.7, 128.5, 128.1, 124.2, 101.2, 100.8, 100.4, 75.2, 73.9, 73.1, 72.8, 72.0, 71.8, 70.0, 68.8, 68.2, 67.0, 61.9, 56.3, 48.4, 32.9, 32.8, 30.3, 29.9, 29.8, 29.7, 29.6, 29.5, 29.3, 28.0, 20.9, 20.85, 20.81, 20.78, 20.71, 20.68 ppm; IR ν[cm⁻¹] = 2926 (m), 2854 (m), 1750 (s), 1656 (w), 1585 (w), 1458 (w), 1436 (w), 1367 (m), 1220 (s), 1168 (m), 1120 (w), 1076 (m), 1041 (s), 985 (w), 929 (w), 860 (w), 803 (w), 741 (w), 697 (w); HRMS (ESI⁺) m/z [M+Na⁺] calcd for C₅₉H₉₈O₂₀SN⁺, 1171.54819 found 1171.54573.

(3*R*,8*E*)-Benzyl-(4-aza-4-methyl-3-(methoxycarbonyl)-5,7-di-oxo-octacosanic acid)-(2'',3'',4'',6''-tetra-*O*-acetyl-β-D-glucopyranosyl)-(1→4)-2',3'-di-*O*-acetyl-1'-*O*-β-D-galactopyranoside) uronate (32)



To a solution of 0.053 g (0.046 mmol, 1.00 eq) **6** and 0.014 g (0.055 mmol, 1.20 eq) **7** in 2.3 mL dry THF at 0 °C were added 12.9 μL (0.092 mmol, 2.00 eq) NEt₃ and 0.012 g (0.055 mmol, 1.20 eq) AgO₂CCF₃ under light exclusion. The reaction was stirred at this temperature for 3 h and then diluted with Et₂O and a 1 M aqueous HCl solution. The aqueous phase was separated and extracted three times with Et₂O. The combined organic phases were washed with brine, dried over Na₂SO₄ and concentrated. The remaining yellow oil was purified by column chromatography (silica, 60/40 cHex/EtOAc) to afford 0.038 g (0.029 mmol, 63%) of the title compound as a pale yellow syrup. The product was used without further purification.

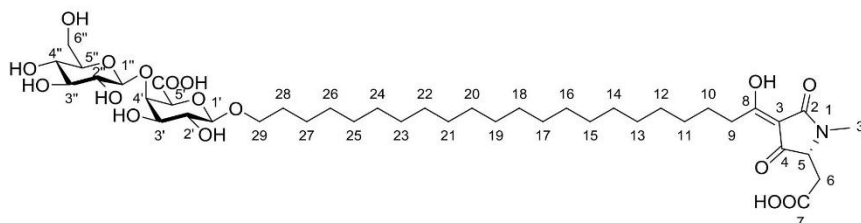
(3*R*)-4-Aza-4-methyl-3-(methoxycarbonyl)-5,7-di-oxo-octacosanic acid)-(2'',3'',4'',6''-tetra-*O*-acetyl-β-D-glucopyranosyl)-(1→4)-2',3'-di-*O*-acetyl-1'-*O*-β-D-galactopyranosiduronic acid (5)



0.075 g 5% palladium on charcoal were added to a solution of 0.069 g (0.053 mmol, 1.00 eq) **32** in 2 mL dry EtOAc at room temperature. The suspension was saturated with H₂ and stirred under 1 bar of H₂ gas for 3.5 h. The reaction mixture was filtered through Celite® and concentrated in vacuo to afford 0.050 g (0.044 mmol, 84%) of the title compound as a colourless syrup. The product was used without further purification. [α]_D²⁵ +24.1 ° (c = 1.00, CHCl₃); ¹H NMR (500 MHz, CDCl₃) δ 5.19 (dd, J=9.5, 9.7 Hz, 1H; 3''-H), 5.13-5.02 (m, 3H; 2-H keto, 2'-H, 4''-H), 4.99-4.81 (m, 3H; 3'-H, 2''-H, 2'''-H), 4.63-4.54 (m, 1H; 24-H^a), 4.50 (d, J=7.9 Hz, 1H; 1''-H), 4.45 (d, J=8.0 Hz, 1H; 1'-H), 4.44 (d, J=2.7 Hz, 1H; 4'-H), 4.15 (s, 1H; 5'-H), 3.94-3.84 (m, 2H; 6''-H^a, 24-H^b), 3.73 (s, 3H; OMe), 3.60 (dt, J=2.7, 9.9 Hz, 1H; 5''-H), 3.55 (s, 1H; 2-H enol), 3.48-3.40 (m, 1H; 6'''-H^b), 3.20 (dd, J=5.7, 16.6 Hz, 1H; 3'''-H^a), 3.05 (s, 3H; N-Me), 2.87 (dd, J=6.6, 16.5 Hz, 1H; 3'''-H^b), 2.53 (dd, J=7.4, 7.6 Hz, 2H; 4-H), 2.14 (s, 3H; CH₃-Ac), 2.12 (s, 3H; CH₃-Ac), 2.11 (s, 3H; CH₃-Ac), 2.02 (s, 6H; CH₃-Ac), 2.00 (s, 3H; CH₃-Ac), 1.63-

1.48 (m, 4H; 5-H, 23-H), 1.63-1.48 (m, 34H, 6-H to 22-H); ^{13}C NMR (125 MHz, CDCl_3) δ 204.9, 204.3, 179.7, 175.0, 173.0, 172.5, 170.6, 170.4, 170.1, 169.9, 169.5, 169.4, 169.3, 168.1, 167.9, 167.8, 101.4, 100.6, 86.4, 76.1, 73.1, 73.0, 72.7, 71.7, 71.3, 70.1, 68.9, 68.3, 61.0, 60.5, 57.0, 53.1, 52.8, 49.5, 49.1, 43.3, 43.1, 36.2, 36.1, 35.3, 33.9, 29.8, 29.74, 29.70, 29.6, 29.51, 29.46, 29.4, 29.2, 26.6, 25.9, 23.59, 23.56, 21.2, 20.9, 20.82, 20.78, 20.75, 20.6 ppm; IR $\nu[\text{cm}^{-1}]$ = 2923 (m), 2853 (m), 1744 (s), 1631 (w), 1494 (w), 1464 (w), 1434 (w), 1367 (m), 1219 (s), 1169 (m), 1123 (m), 1084 (m), 1040 (s), 917 (w), 732 (w), 601 (w); HRMS (ESI $^+$) m/z $[\text{M}+\text{Na}^+]$ calcd for $\text{C}_{54}\text{H}_{85}\text{O}_{24}\text{NNa}^+$ 1154.53537 found 1154.53577.

Ancorinoside A (1)



A solution of 0.050 g (0.044 mmol, 1.00 eq) **5** in 2.5 mL dry MeOH at room temperature was treated with 0.012 g (0.221 mmol, 5.00 eq) NaOMe and stirred for 1 h at this temperature. The reaction mixture was acidified by adding DOWEX 50WX8-100® resin, filtered, and concentrated in vacuo. The residue thus obtained was recrystallized from MeOH to afford 0.018 g (0.021 mmol, 48%) of ancorinoside A (**1**) as a beige-coloured solid. m_p = 173-174 °C; $[\alpha]_D^{25} +7.0^\circ$ (c = 0.90, MeOH); ^1H NMR (500 MHz, MeOD) δ 4.48 (d, $J=7.8$ Hz, 1H; 1''-H), 4.34 (dd, $J=0.6, 2.9$ Hz, 1H; 4'-H), 4.28 (d, $J=7.6$ Hz, 1H; 1'-H), 4.24 (d, $J=0.6$ Hz, 1H; 5'-H), 4.06 (dd, $J=4.4, 4.7$ Hz, 1H; 5-H), 3.94 (dt, $J=6.8, 9.4$ Hz, 1H; 29-H a), 3.85 (dd, $J=2.3, 12.0$ Hz, 1H; 6''-H a), 3.67-3.61 (m, 2H; 3'-H, 6''-H b), 3.59-3.52 (m, 2H; 29-H b , 2'-H), 3.34-3.27 (m, 2H; 4''-H, 5''-H), 3.24-3.18 (m, 2H; 2''-H, 3''-H), 2.96 (s, 3H; 30-H), 2.91 (dd, $J=4.7, 17.1$ Hz, 1H; 6-H a), 2.88-2.76 (m, 3H; 6-H b , 9-H), 1.72-1.59 (m, 4H; 10-H, 28-H), 1.47-1.14 ppm (m, 34H; 11-H to 27-H); ^{13}C NMR (125 MHz, MeOD) δ 188.9, 174.8, 179.9, 172.6, 106.7, 105.5, 103.4, 81.7, 78.7, 78.7, 76.4, 75.7, 75.4, 73.3, 72.0, 71.9, 65.2, 63.3, 35.2, 34.1, 31.5, 31.2, 31.1, 31.0, 27.9, 27.8, 27.6 ppm; IR $\nu[\text{cm}^{-1}]$ 3335 (br m), 2920 (s), 2850 (s), 1747 (m), 1714 (m), 1702 (m), 1677 (m), 1646 (m), 1633 (m), 1492 (w), 1470 (w), 1449 (w), 1411 (w), 1365 (w), 1256 (w), 1226 (w), 1202 (w), 1161 (w), 1076 (m), 1031 (m), 991 (w), 947 (w), 903 (w), 757 (w), 719 (w), 683 (w); HRMS (ESI $^+$) m/z $[\text{M}+\text{Na}^+]$ calcd for $\text{C}_{41}\text{H}_{69}\text{O}_{17}\text{NNa}^+$, 870.44577 found 870.44375; UV (MeOH) 202 nm, 232 nm, 283 nm; CD (MeOH) 285 nm ($\Delta\epsilon$ +0.46), 240 nm ($\Delta\epsilon$ +4.21).

Table 1 Comparison of ^1H and ^{13}C NMR shifts of natural and synthetic ancorinoside A (**1**).

| position | natural ancorinoside A in MeOD δ_{H} (mult., J[Hz], intg.) | synthetic ancorinoside A in MeOD δ_{H} (mult., J[Hz], intg.) | natural ancorinoside A in MeOD δ_{C} [ppm] | synthetic ancorinoside A in MeOD δ_{C} [ppm] |
|----------|--|--|---|---|
| 1 | -- | -- | -- | -- |
| 2 | -- | -- | 175.1 | 174.8 ^b |
| 3 | -- | -- | 103.8 | 103.4 ^b |
| 4 | -- | -- | 197.0 | nd |
| 5 | 4.05 (br s, 1H) | 4.06 (dd, 4.6/4.4, 1H) | 64.7 | 65.2 ^b |
| 6a | 2.80 (m, 1H) | 2.88-2.76 (m, 1H) | 36.0 | 35.2 |
| 6b | 2.90 (dd, 16.5/4.6, 1H) | 2.91 (dd, 17.1/4.7, 1H) | 36.0 | 35.2 |
| 7 | -- | -- | 174.5 | 173.9 |
| 8 | -- | -- | 188.9 | 188.9 ^b |
| 9 | 2.80 (m, 2H) | 2.88-2.73 (m, 2H) | 35.8 | 34.1 |
| 10 | 1.65 (m, 2H) | 1.72-1.59 (m, 2H) | 27.9 | 27.8 |
| 11 | 1.30 (m, 2H) | 1.47-1.14 (m, 2H) | 31.5 | 31.5 |
| 12-26 | 1.30 (m, 2H) | 1.47-1.14 (m, 32H) | 31.5 | 31.5 |
| 27 | 1.40 (m, 2H) | 1.44-1.36 (m, 2H) | 27.9 | 27.9 |
| 28 | 1.63 (m, 2H) | 1.72-1.59 (m, 2H) | 31.5 | 31.5 |
| 29a | 3.54 (m, 1H) | 3.58-3.52 (m, 1H) | 72.1 | 72.0 |
| 29b | 3.94 (dt, 9.2/6.4, 1H) | 3.94 (dt, 9.4/6.8, 1H) | 72.1 | 72.0 |
| 30 | 2.96 (s, 3H) | 2.96 (s, 3H) | 26.9 | 27.6 |
| 1' | 4.27 (d, 7.3, 1H) | 4.28 (d, 7.6, 1H) | 105.5 | 105.5 |
| 2' | 3.56 (m, 1H) | 3.59-3.52 (m, 1H) | 73.4 | 73.3 |
| 3' | 3.63 (m, 1H) | 3.67-3.61 (m, 1H) | 75.8 | 75.7 |
| 4' | 4.33 (d, 2.8, 1H) | 4.34 (dd, 2.9/0.6, 1H) | 81.7 | 81.7 |
| 5' | 4.23 (s, 1H) | 4.24 (d, 0.6, 1H) | 75.7 | 75.4 |
| 6' | -- | -- | 173.0 | 172.6 |
| 1'' | 4.47 (d, 7.3, 1H) | 4.48 (d, 7.8, 1H) | 106.7 | 106.7 |
| 2'' | 3.22 (m, 1H) | 3.24-3.18 (m, 1H) | 76.4 | 76.4 |
| 3'' | 3.35 (m, 1H) | 3.24-3.18 (m, 1H) ^a | 78.8 | 78.7 |
| 4'' | 3.30 (m, 1H) | 3.34-3.27 (m, 1H) ^a | 72.1 | 71.9 |
| 5'' | 3.20 (m, 1H) | 3.34-3.27 (m, 1H) ^a | 78.7 | 78.7 |
| 6''a | 3.65 (m, 1H) | 3.67-3.61 (m, 1H) | 63.4 | 63.3 |
| 6''b | 3.84 (dd, 11.9/1.8, 1H) | 3.85 (dd, 12.0/2.3, 1H) | 63.4 | 63.3 |

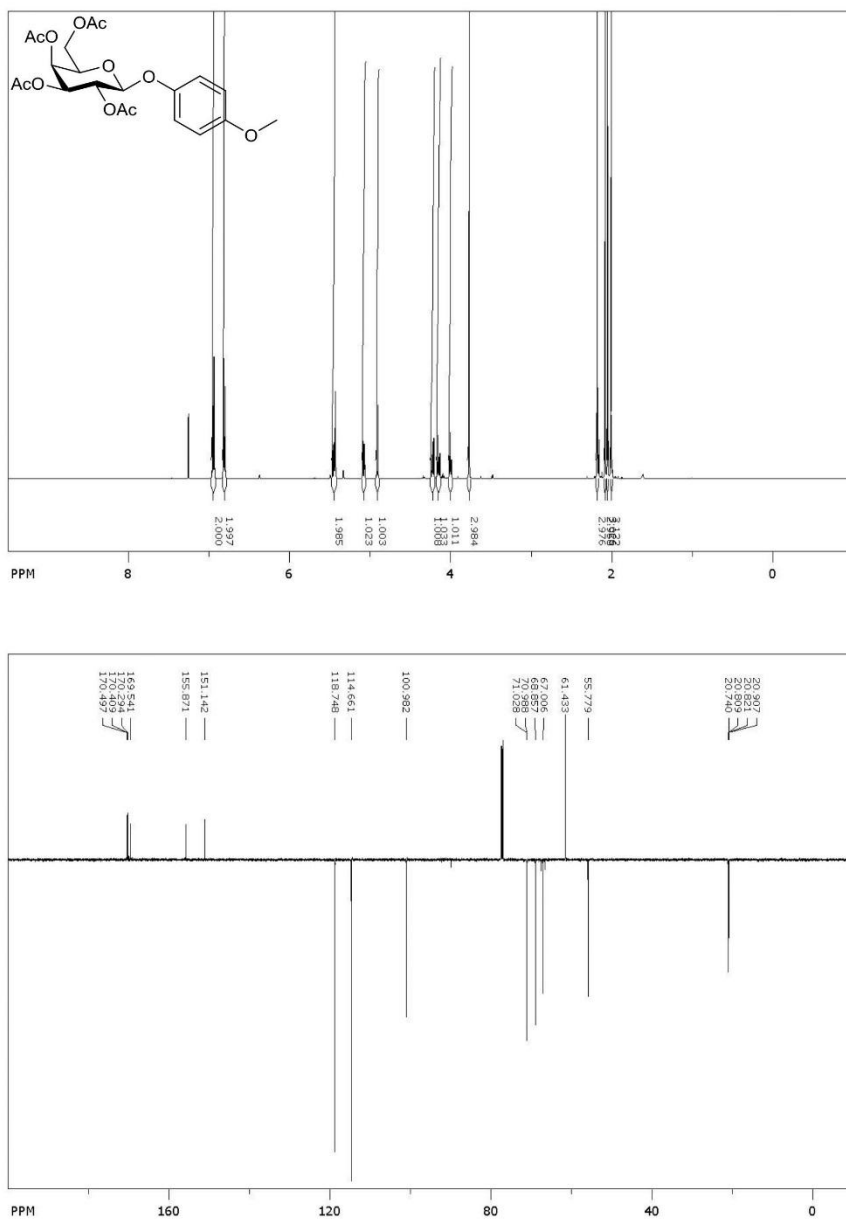
^a Assigned from HSQC cross peak. ^b Assigned from HMBC cross peak. nd not determined

References

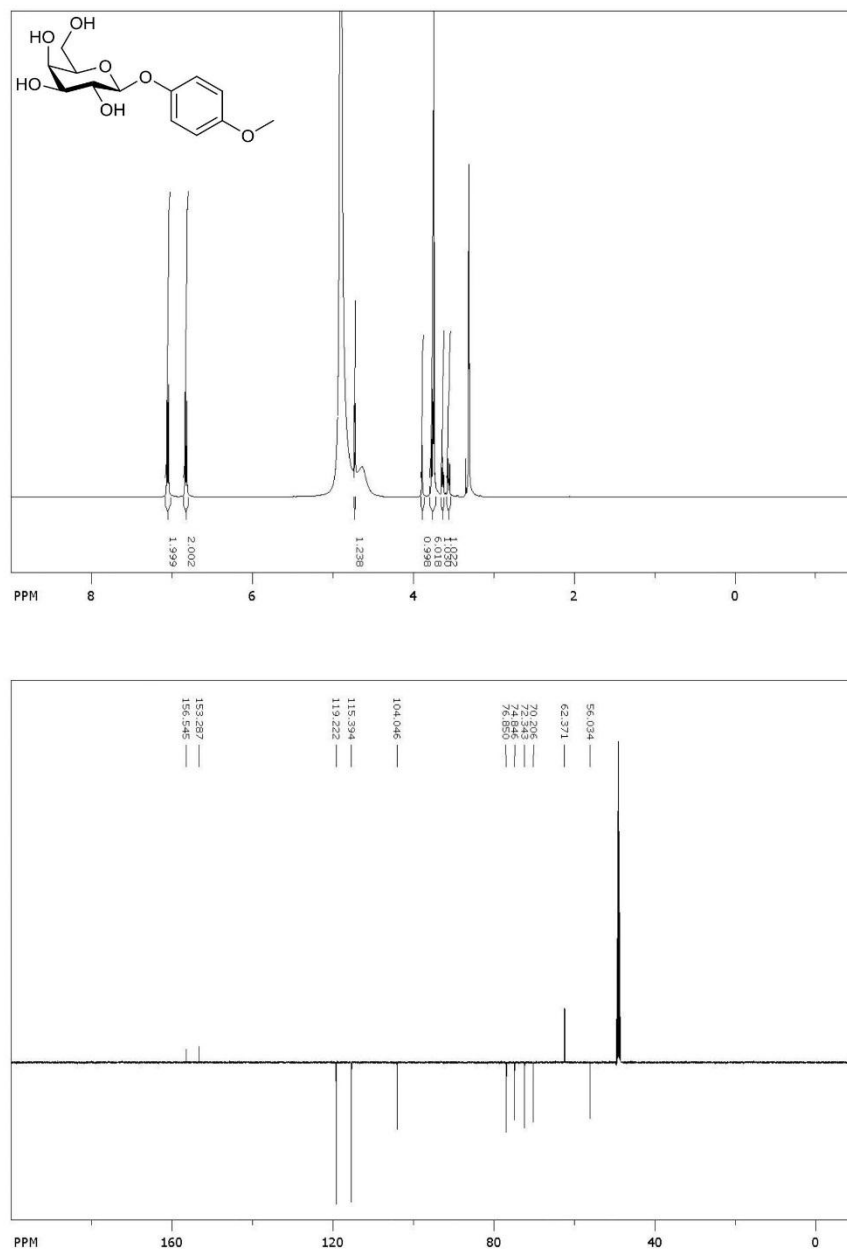
- [1] J. Li, W. Li, B. Yu, *Org. Biomol. Chem.* **2013**, *11*, 4971-4974.
- [2] J. M. Pons, A. Dumont, G. Sautejeau, E. Fugier, A. Baron, S. Dukan, B. Vauzeilles, *Angew. Chem. Int. Ed.* **2014**, *53*, 1275-1278.
- [3] Z. Zhang, G. Magnusson, *J. Org. Chem.* **1996**, *61*, 2383-2393.
- [4] C. E. Martin, M. W. Weishaupt, P. H. Seeberger, *Chem. Commun.* **2011**, *47*, 10260-10262.
- [5] Y. Gao, J. Z. Vlahakis, W. A. Szarek, I. Brockhausen, *Bioorg. Med. Chem.* **2013**, *21*, 1305-1311.
- [6] T. Suzuki, T. Yamamoto, Y. Tezuka, *J. Am. Chem. Soc.* **2014**, *136*, 10148-10155.
- [7] S. V. Ley, P. R. Woodward, *Tetrahedron Lett.* **1987**, *28*, 345-346.
- [8] O. Hartmann, M. Kalesse, *Angew. Chem. Int. Ed.* **2014**, *53*, 7335-7338.
- [9] A. P. Mehrotra, K. L. Webster, D. Gani, *J. Chem. Soc., Perkin Trans. 1*, **1997**, 2495-2511.
- [10] S. Ohta, E. Ohta, S. Ikegami, *J. Org. Chem.* **1997**, *62*, 6452-6453.

NMR Spectra

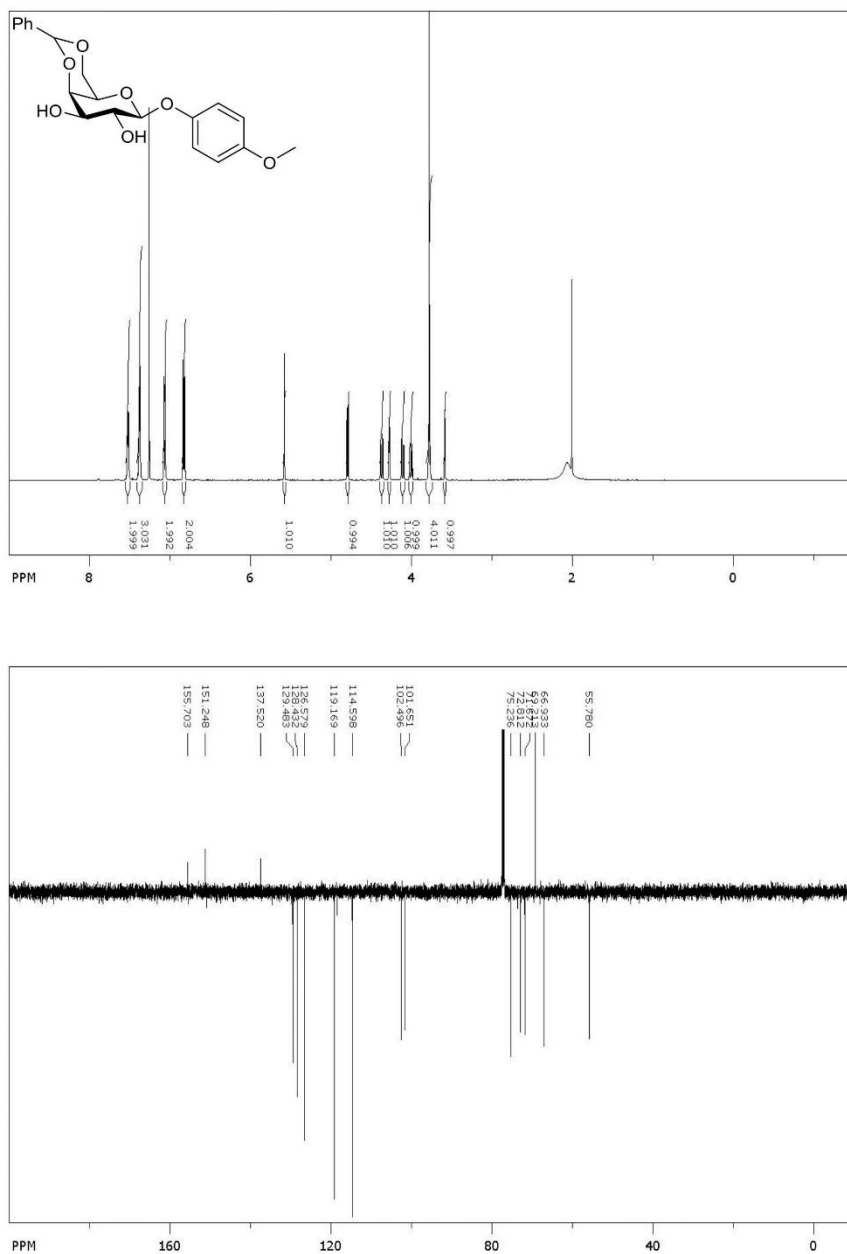
para-Methoxyphenyl-2,3,4,6-tetra-O-acetyl-1-O- β -D-galactopyranoside (**20**)



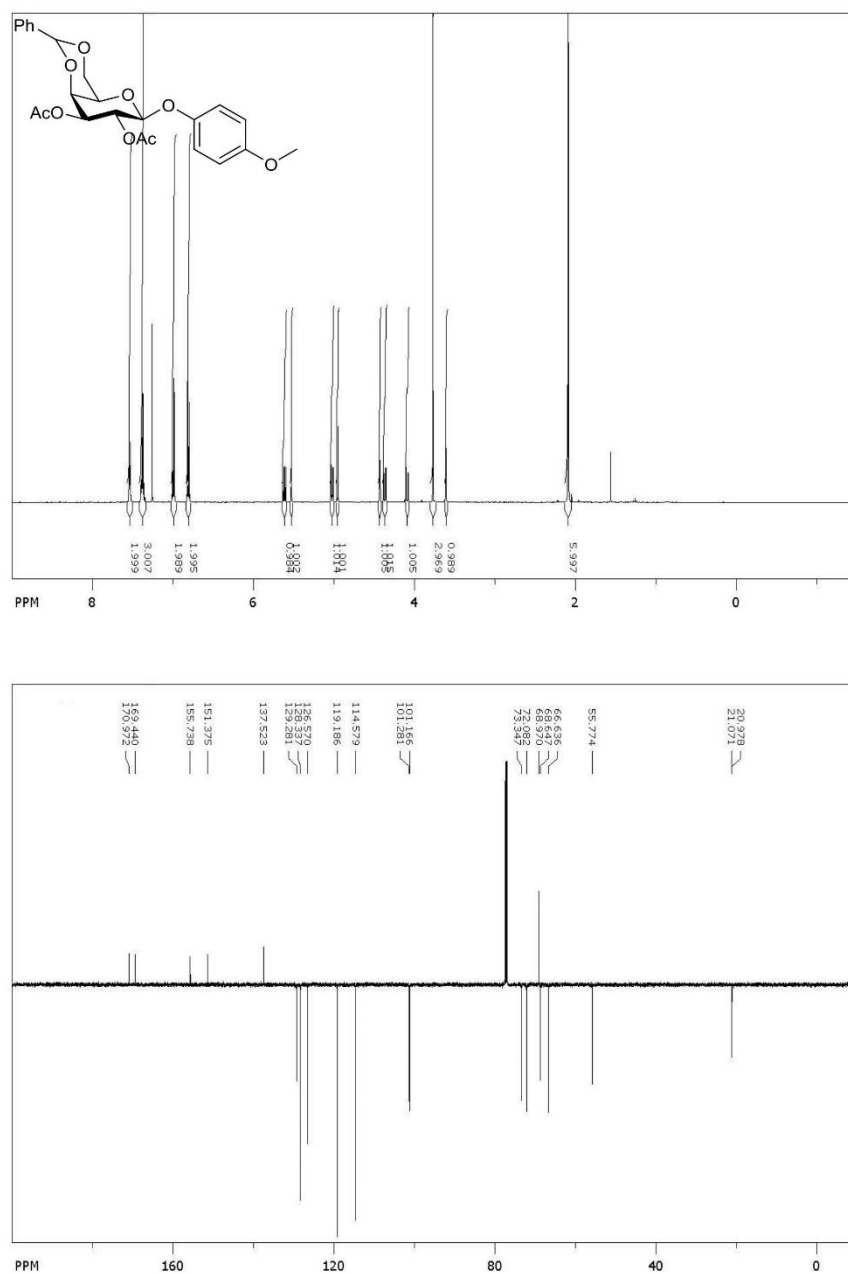
para-Methoxyphenyl-1-O- β -D-galactopyranoside (**21**)



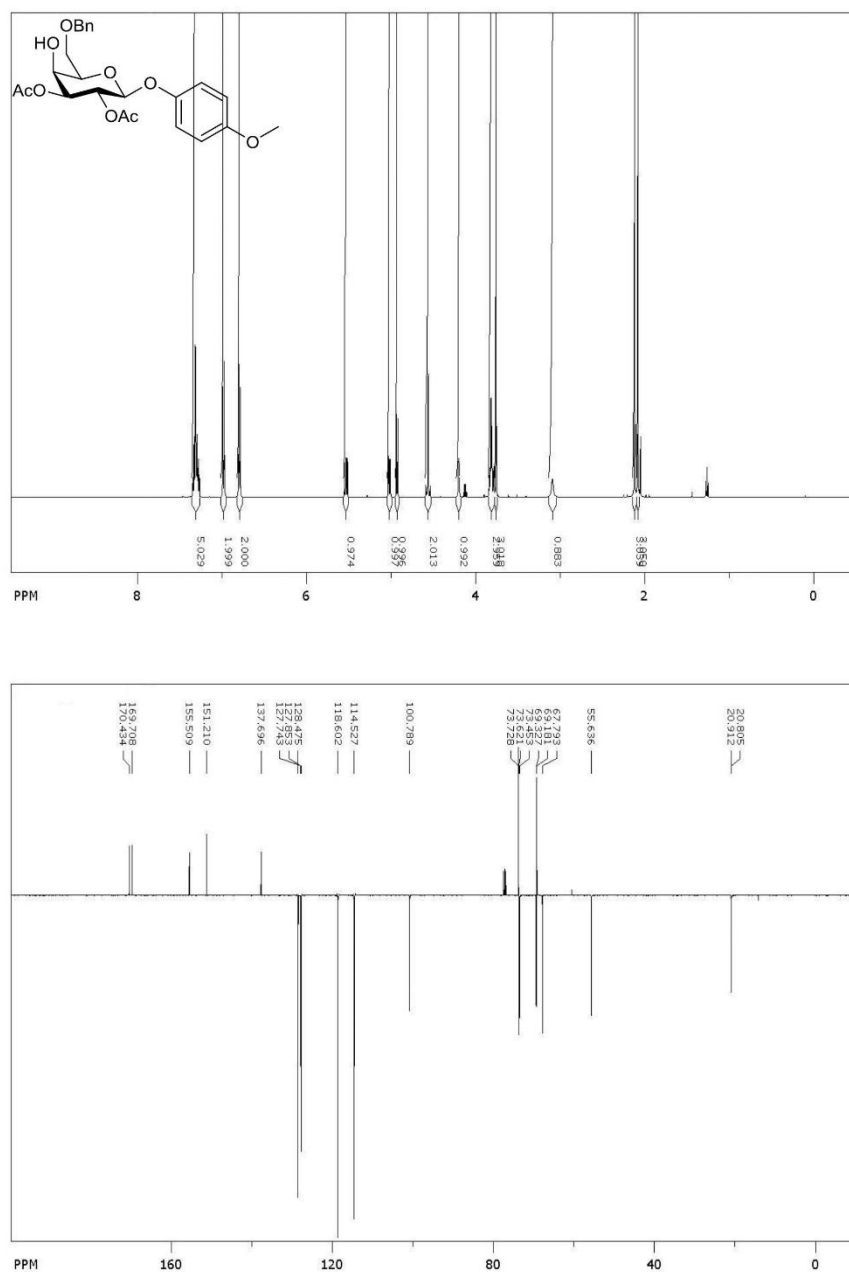
para-Methoxyphenyl-4,6-O-benzylidene-1-O- β -D-galactopyranoside (**22**)



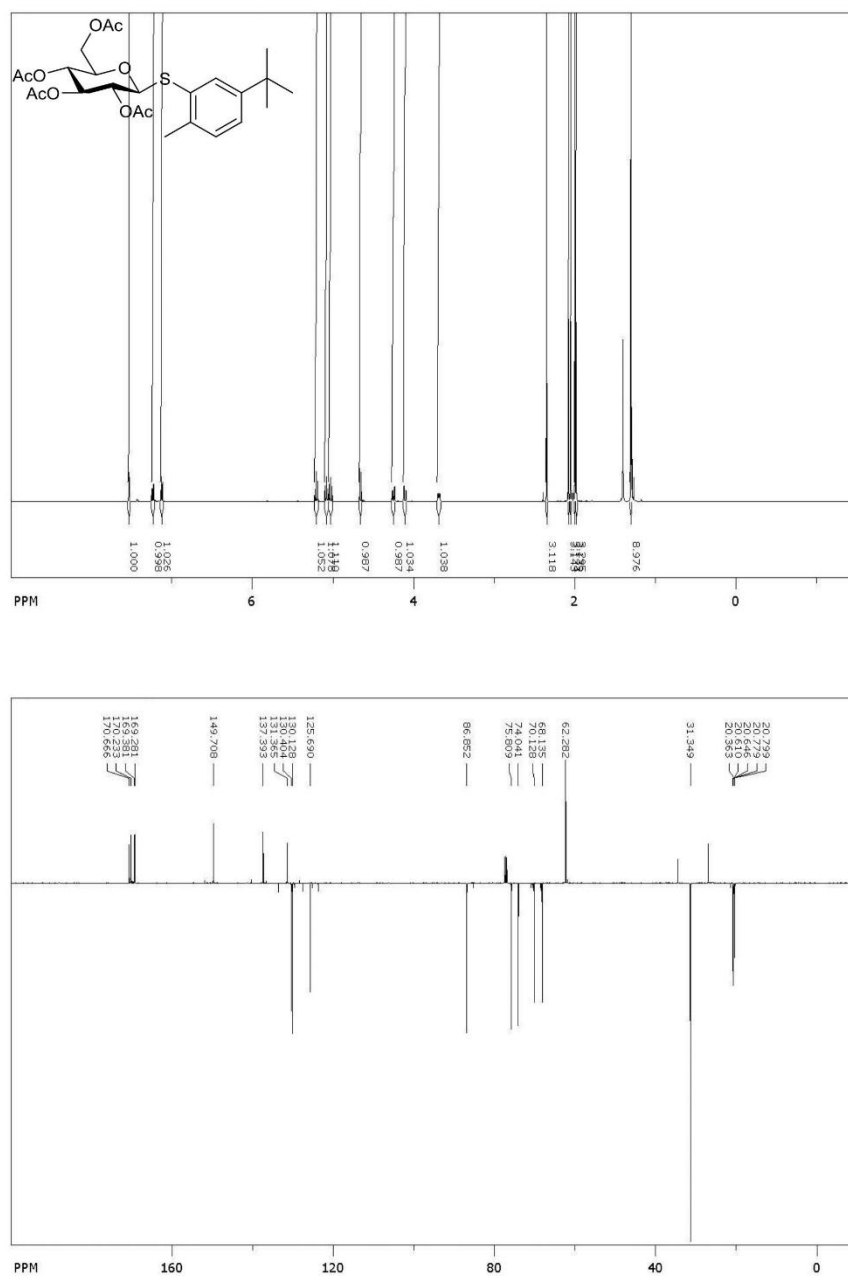
para-Methoxyphenyl-2,3-di-O-acetyl-4,6-benzylidene-1-O- β -D-galactopyranoside (**23**)

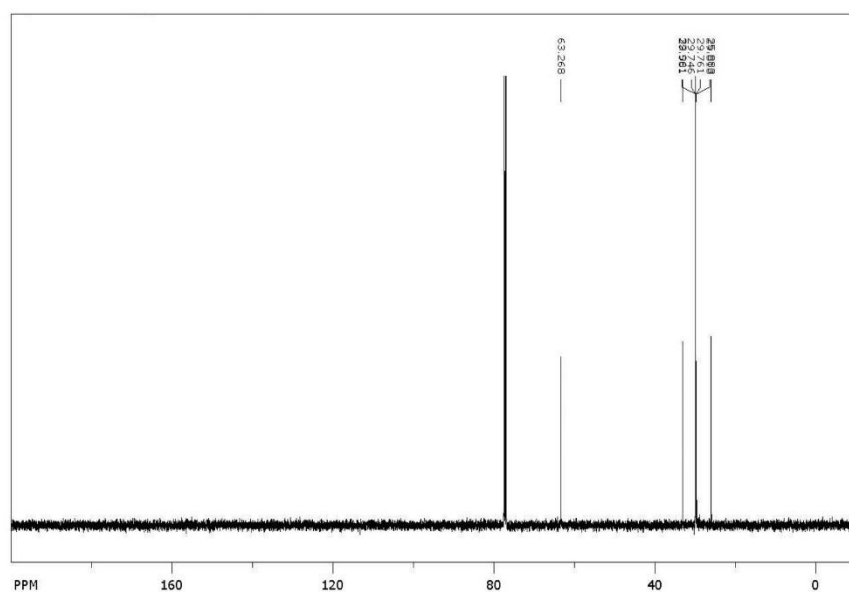
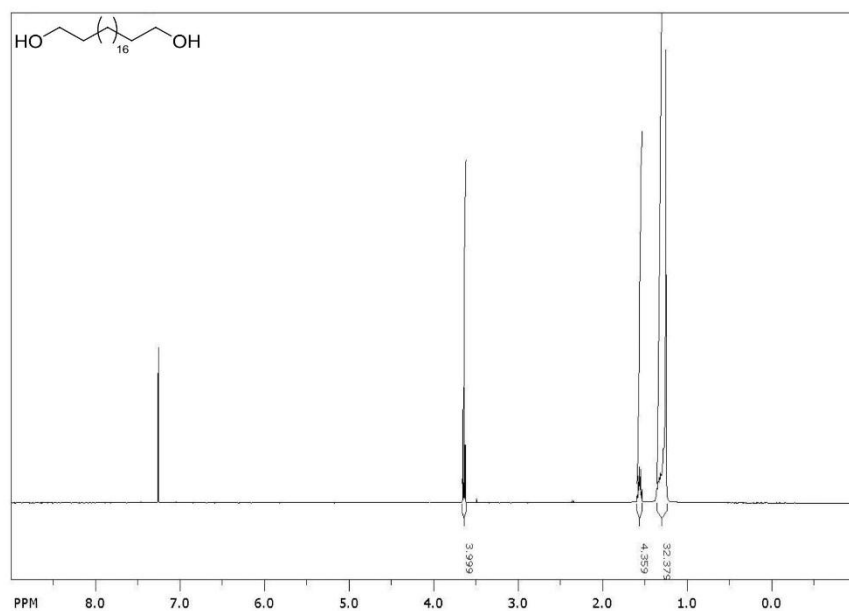


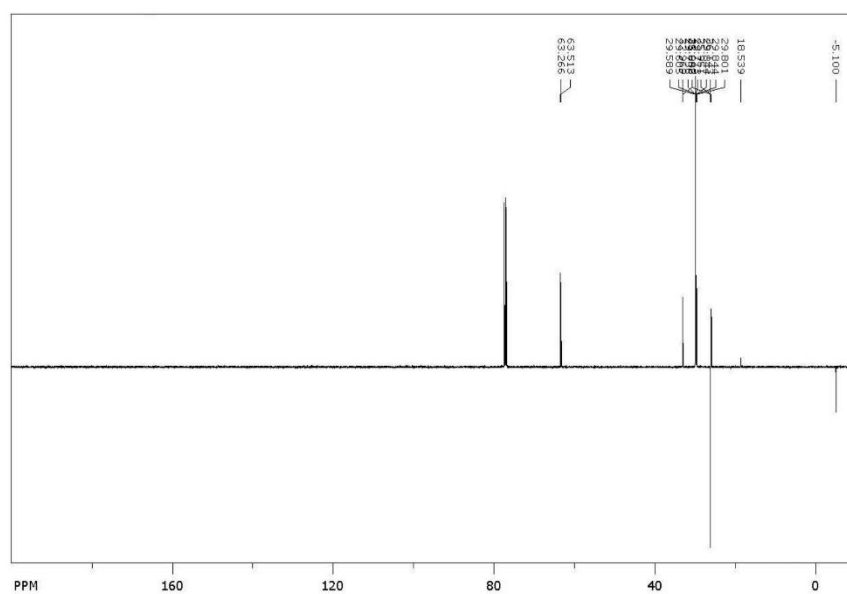
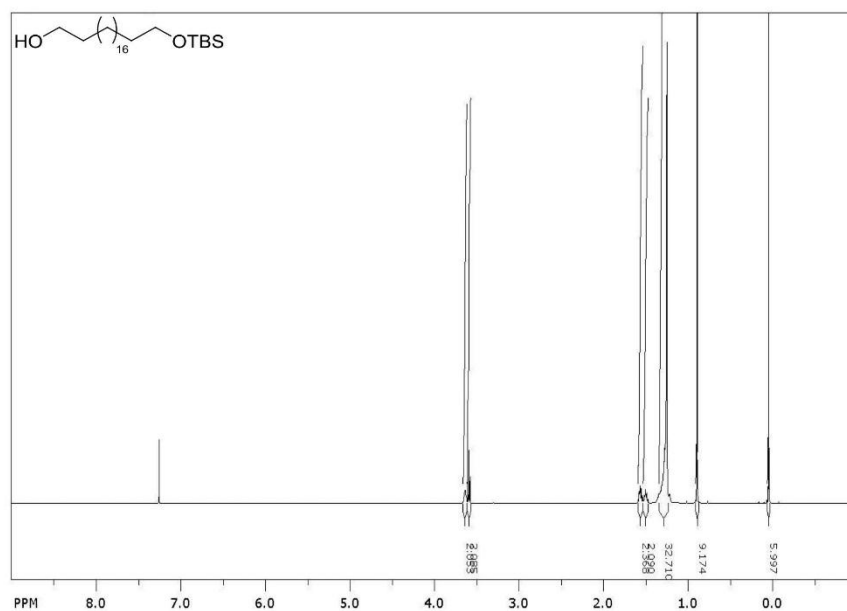
para-Methoxyphenyl-2,3-di-O-acetyl-6-O-benzyl-1-O- β -D-galactopyranoside (**13**)



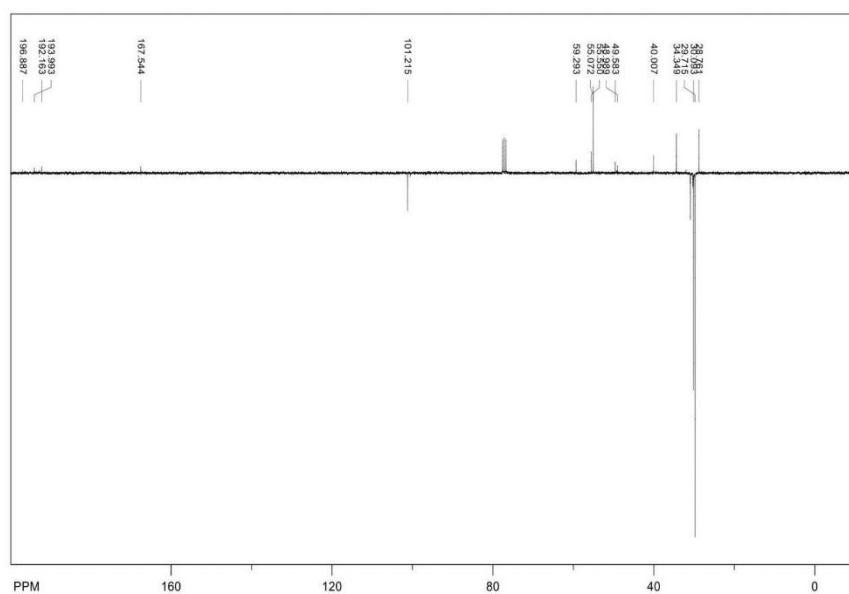
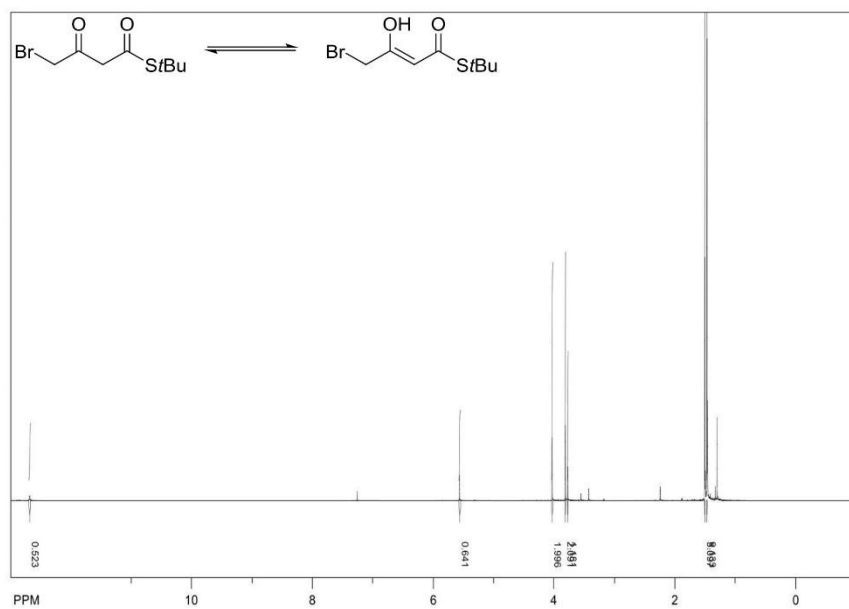
(2-Methyl-5-*tert*-butyl-phenyl)-2,3,4,6-tetra-*O*-acetyl-1-*S*- β -D-thio-glucopyranoside (**12**)



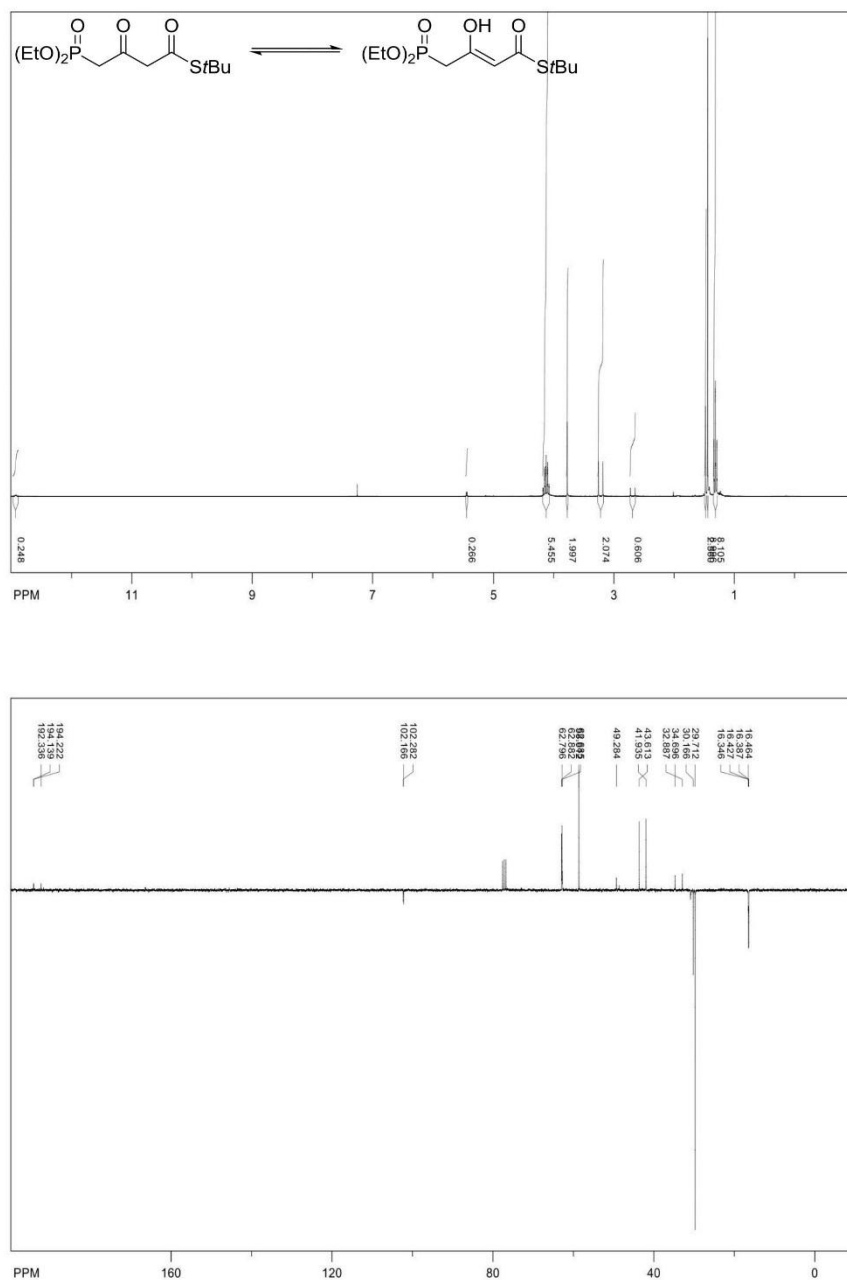
Eicosan-1,20-diol (**18**)

1-[(*tert*-Butyldimethylsilyl)oxy]-20-Eicosandiol (**11**)

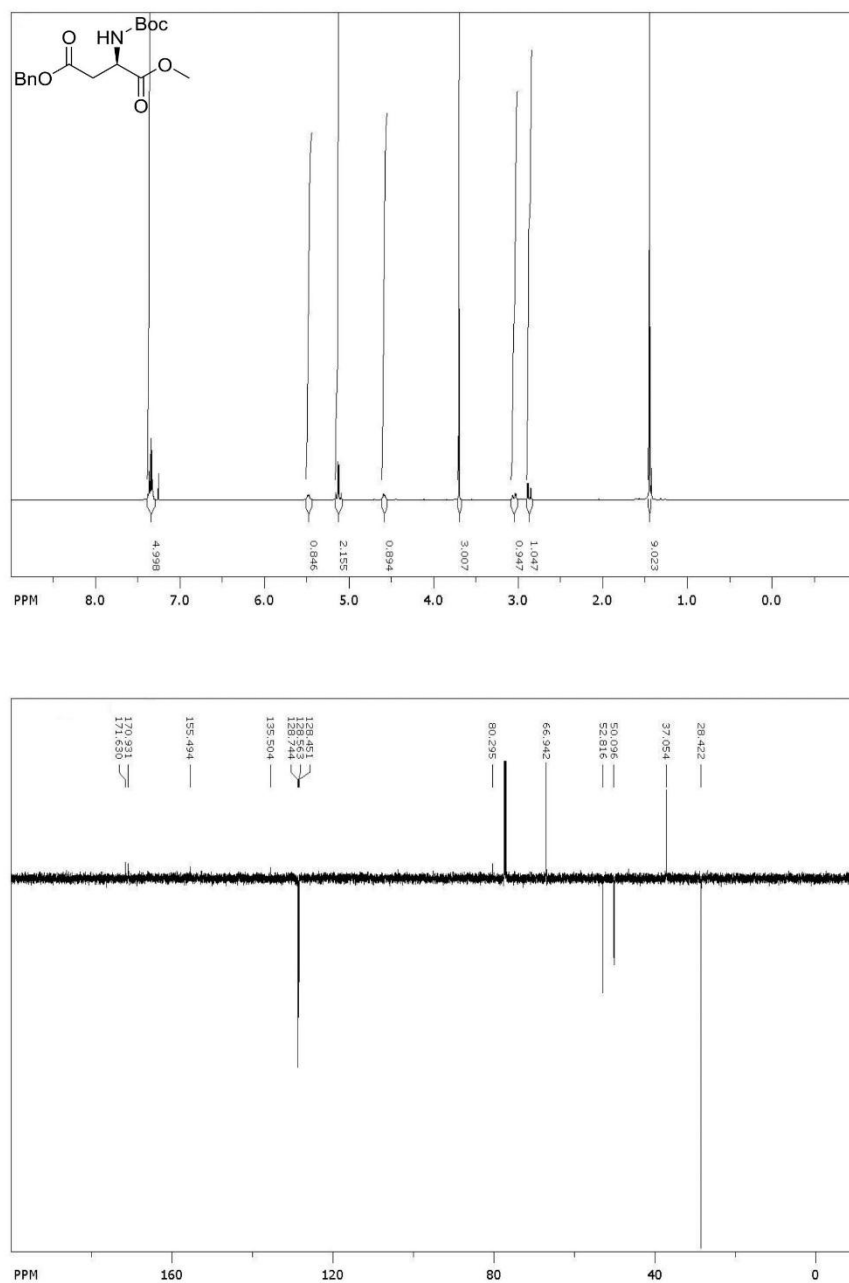
S-tert-Butyl 4-bromo-3-oxo-butanethioate



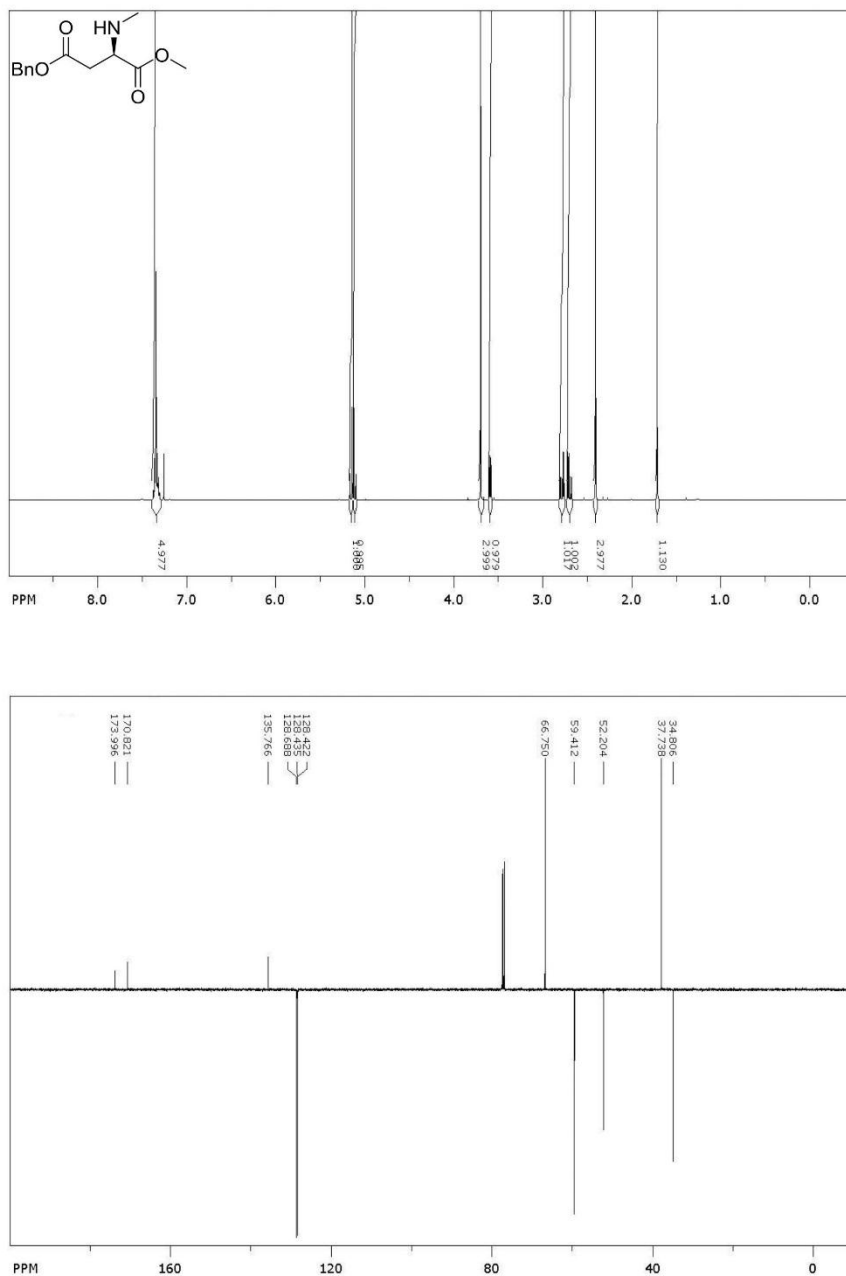
S-*tert*-Butyl 4-(diethoxyphosphono)-3-oxobutanethioate (**9**)



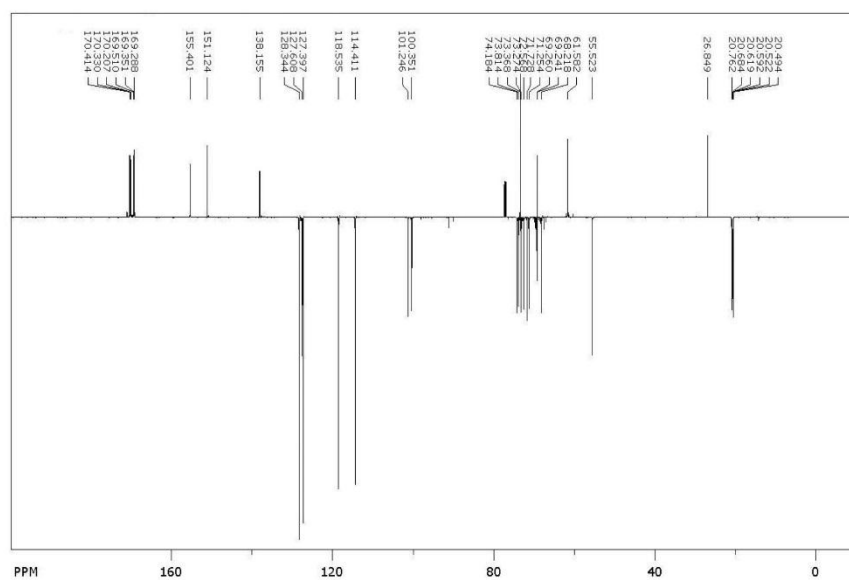
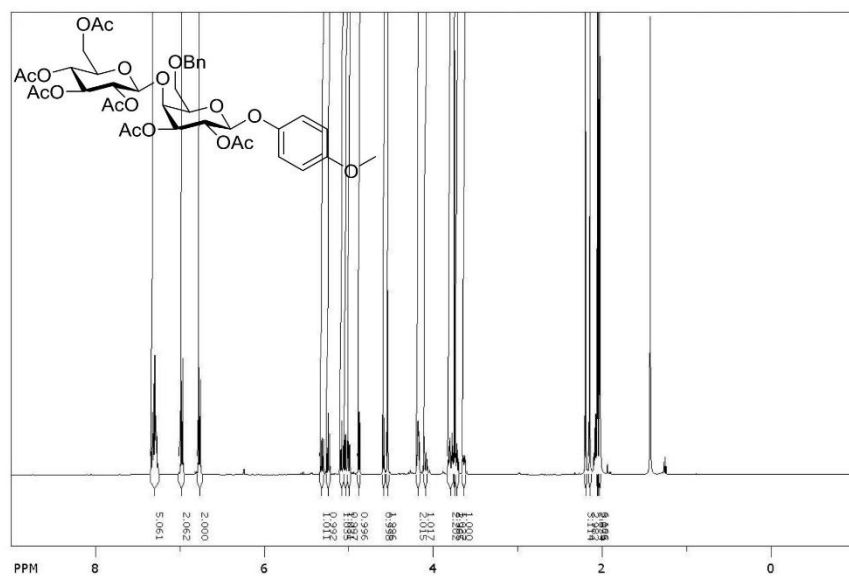
D-N-Boc-Asp(OBn)-OMe (**15**)



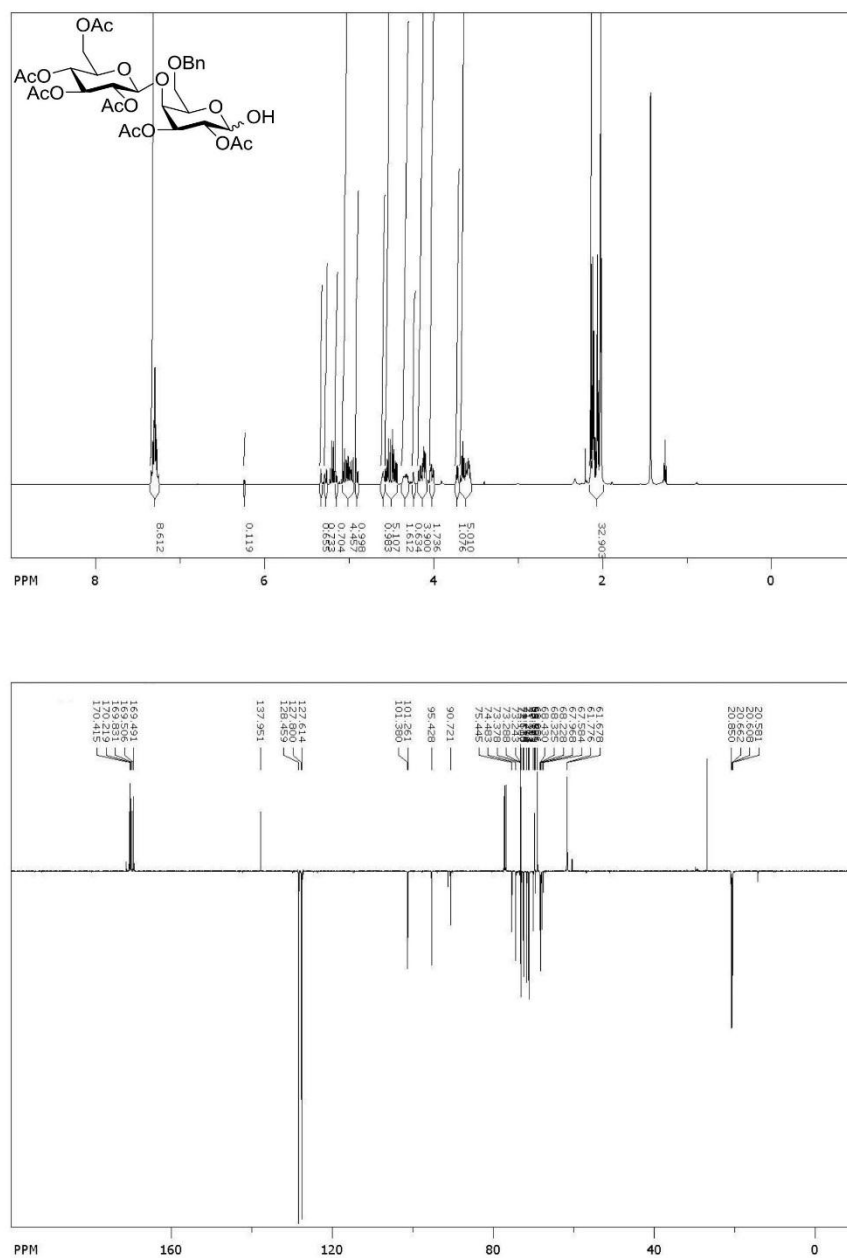
D-N-Me-Asp(OBn)-OMe (7)



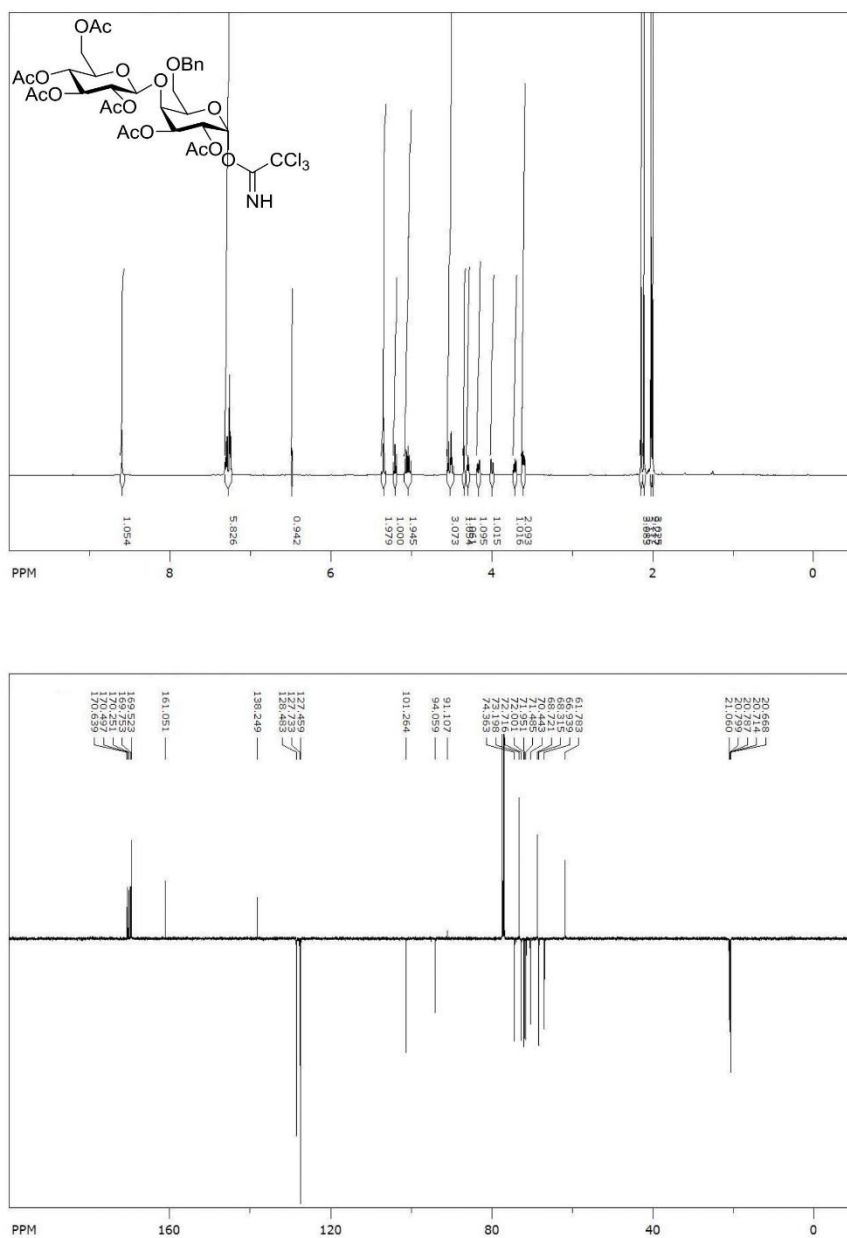
para-Methoxyphenyl-(2'',3'',4'',6''-tetra-O-acetyl- β -D-glucopyranosyl)-(1 \rightarrow 4)-2',3'-di-O-acetyl-6'-O-benzyl-1'-O- β -D-galactopyranoside (**24**)



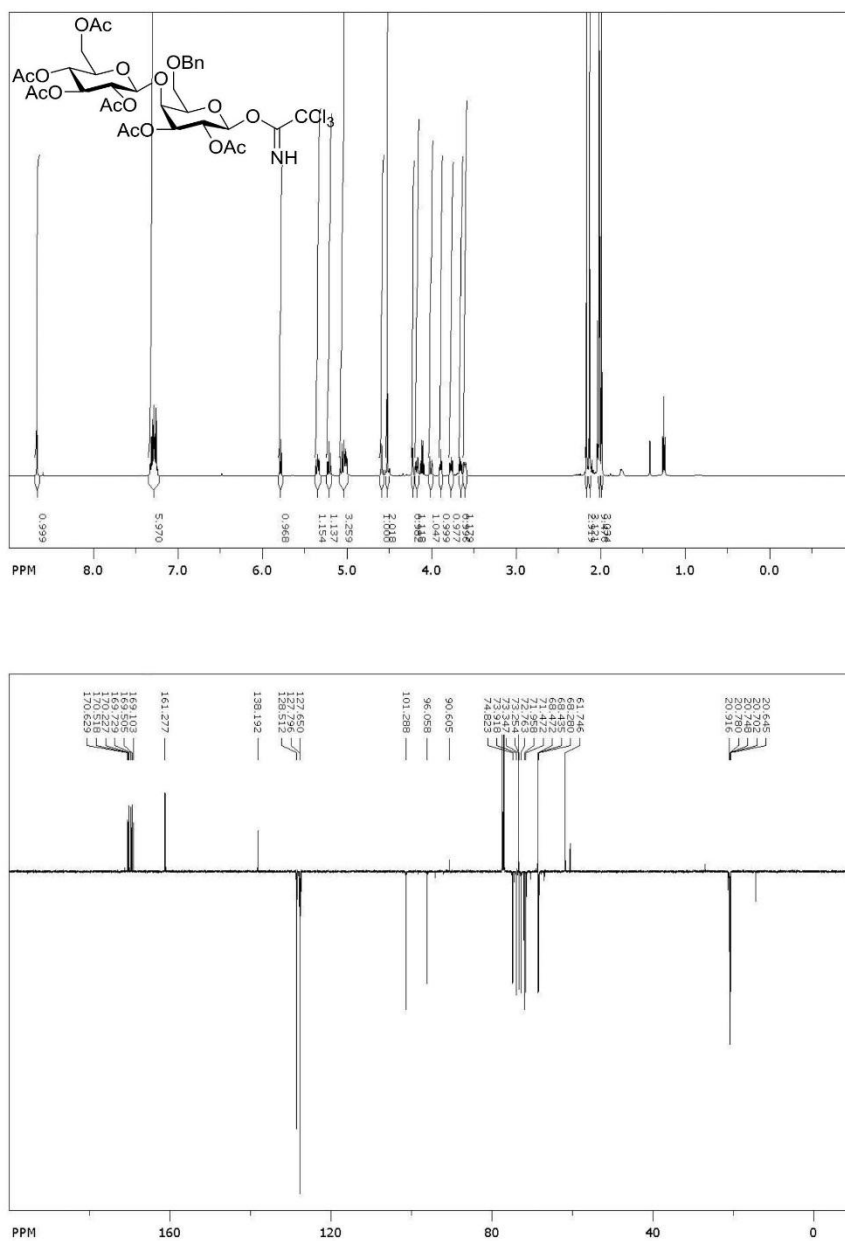
(2'',3'',4'',6''-tetra-O-Acetyl-β-D-glucopyranosyl)-(1→4)-2',3'-di-O-acetyl-6'-O-benzyl-D-galactopyranoside (**25**)



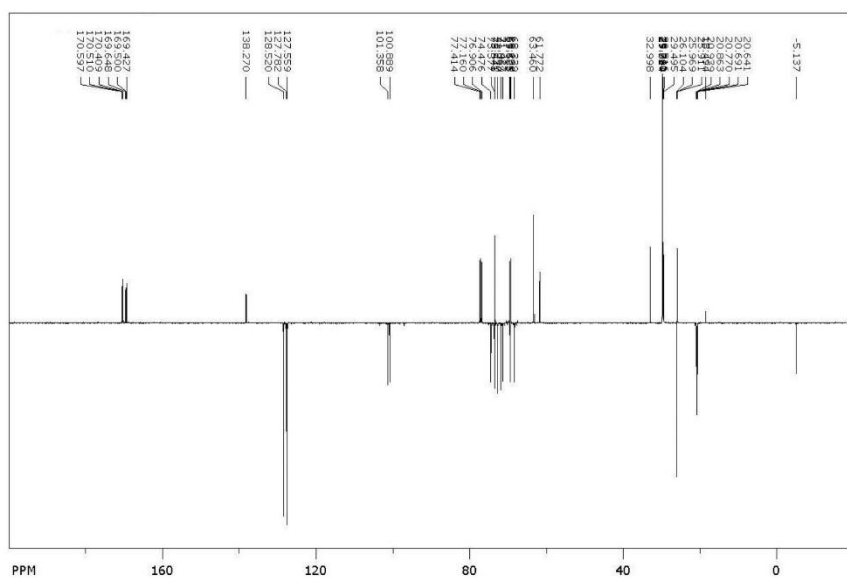
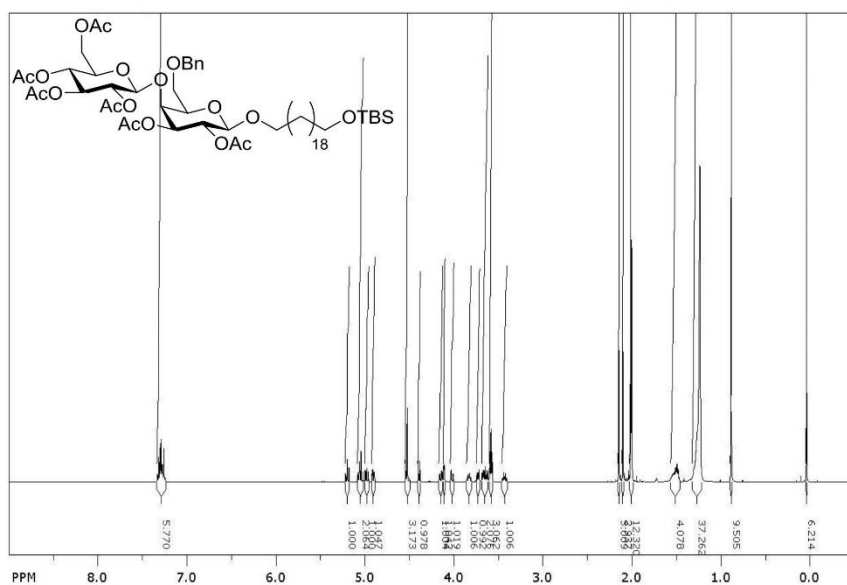
(2'',3'',4'',6''-tetra-O-Acetyl- β -D-glucopyranosyl)-(1 \rightarrow 4)-2'',3''-di-O-acetyl-6''-O-benzyl-1'-O- α -D-galactopyranosyl-trichloroacetimidate (**10a**)



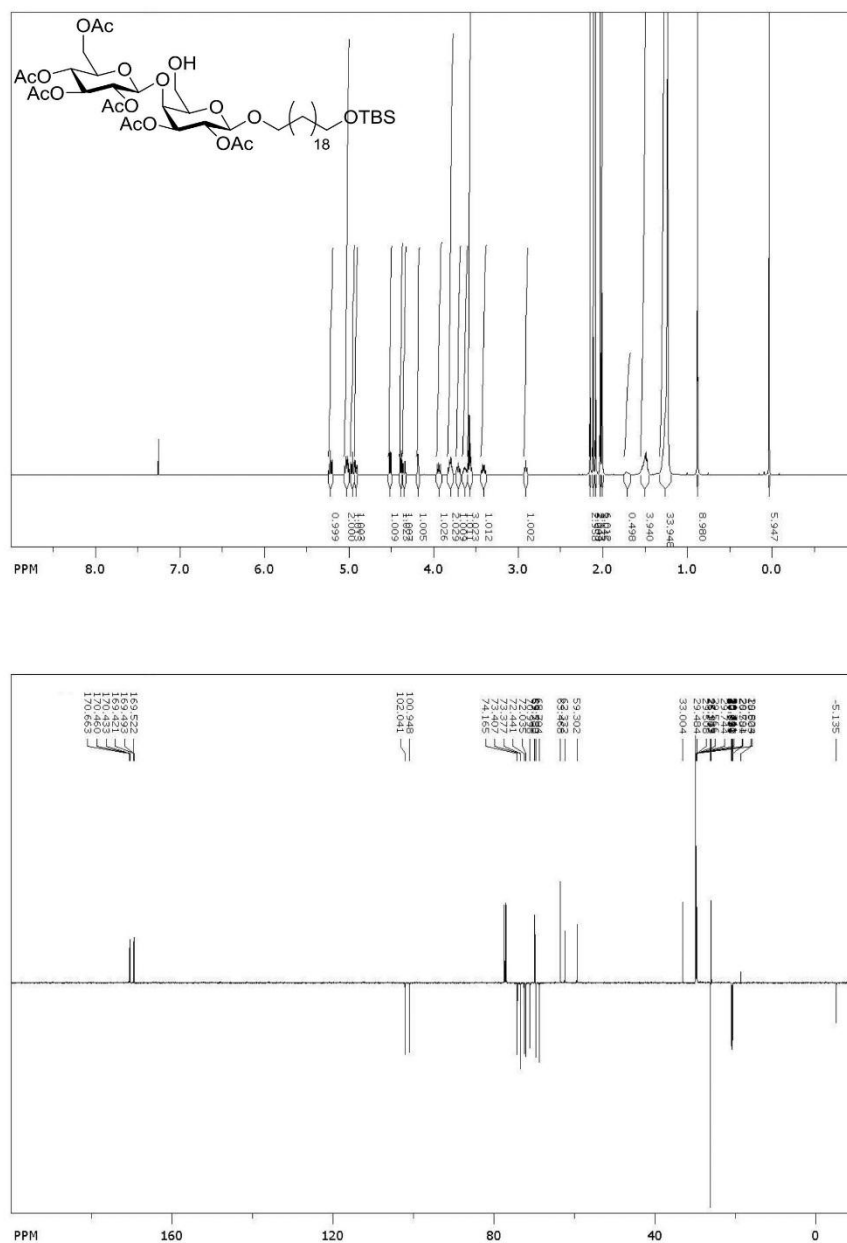
(2'',3'',4'',6'')-tetra-O-Acetyl- β -D-glucopyranosyl)-(1 \rightarrow 4)-2',3'-di-O-acetyl-6'-O-benzyl-1'-O- β -D-galactopyranosyl-trichloroacetimidate (**10b**)



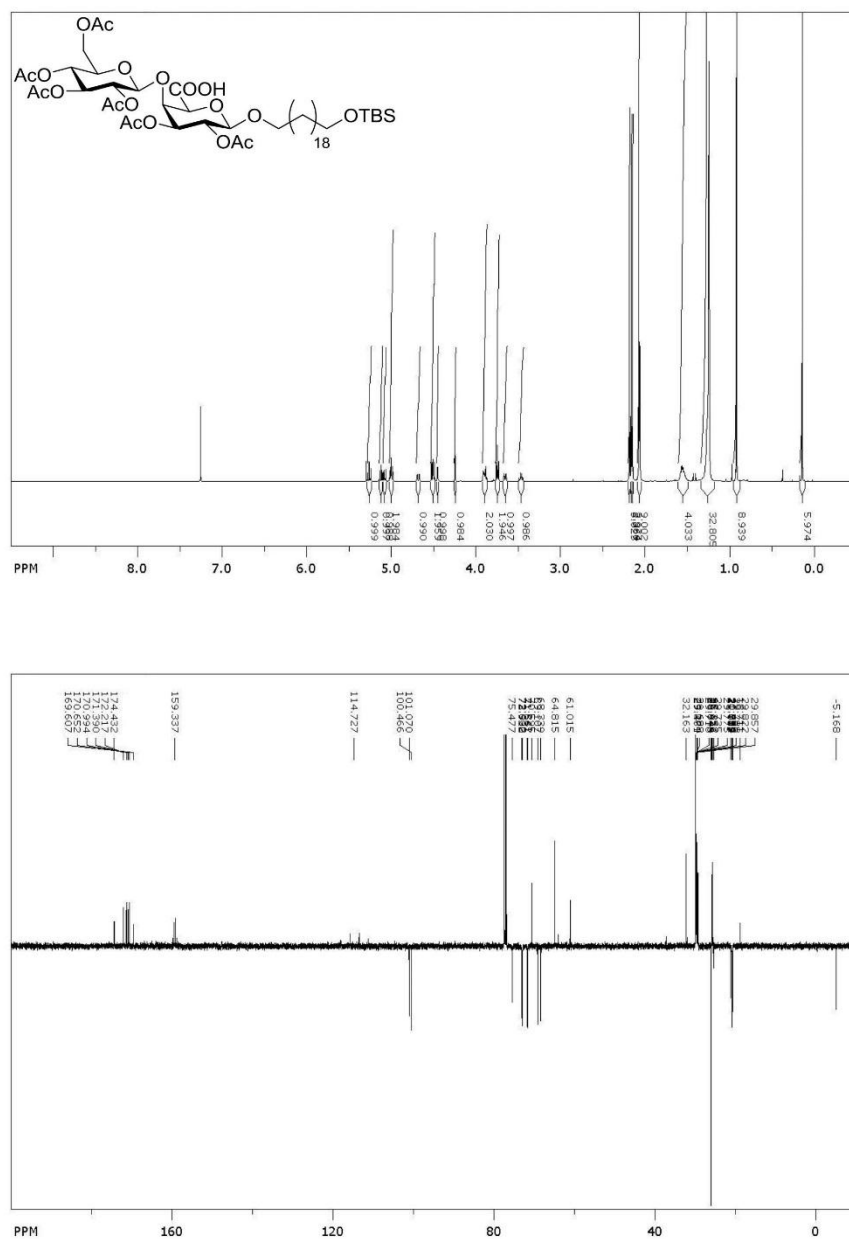
(1-(*tert*-Butyl-dimethyl-silyloxy)-eicosyl)-(2'',3'',4'',6'')-tetra-*O*-acetyl- β -D-glucopyranosyl)-(1 \rightarrow 4)-2',3'-di-*O*-acetyl-6'-*O*-benzyl-1'-*O*- β -D-galactopyranoside (**27**)



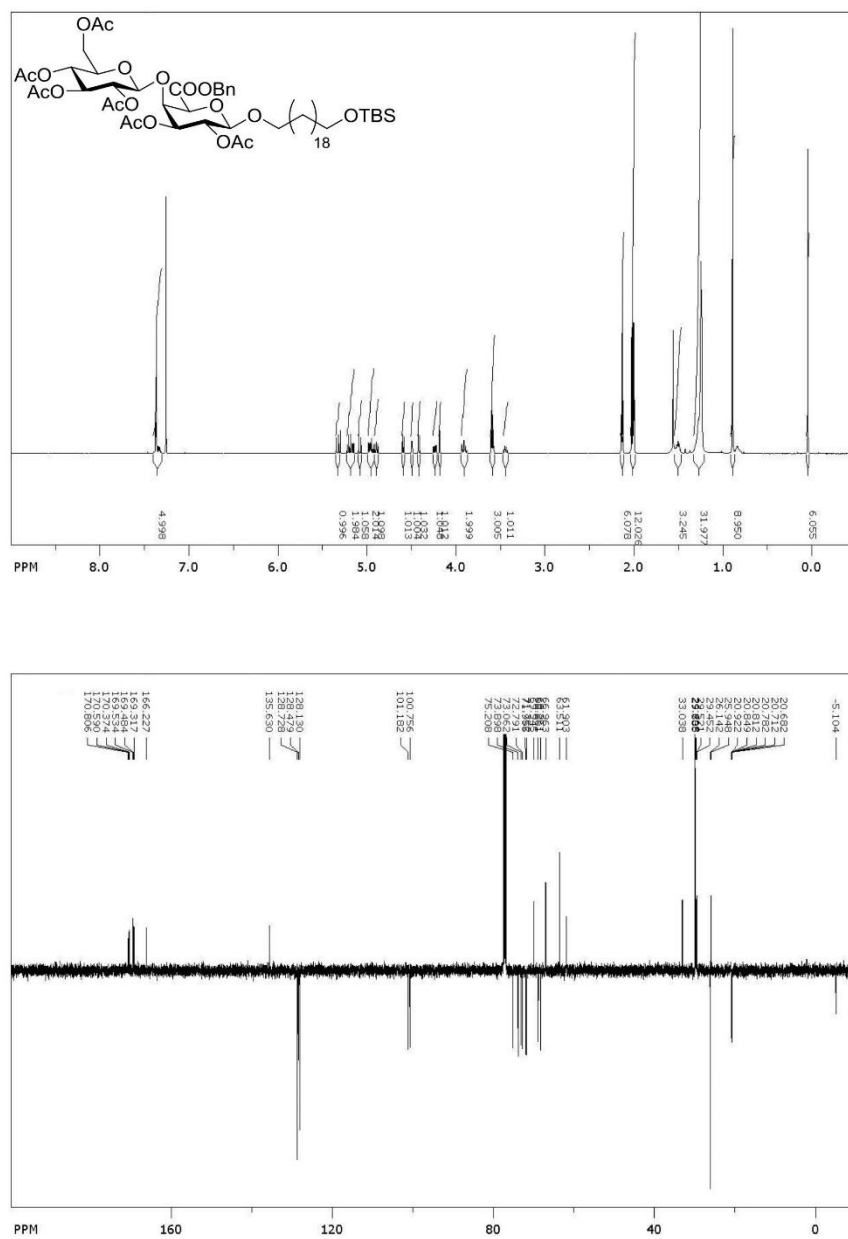
(1-(*tert*-Butyl-dimethyl-silyloxy)-eicosyl)-(2'',3'',4'',6''-tetra-O-acetyl- β -D-glucopyranosyl)-(1 \rightarrow 4)-2',3'-di-O-acetyl-1'-O- β -D-galactopyranoside (**28**)

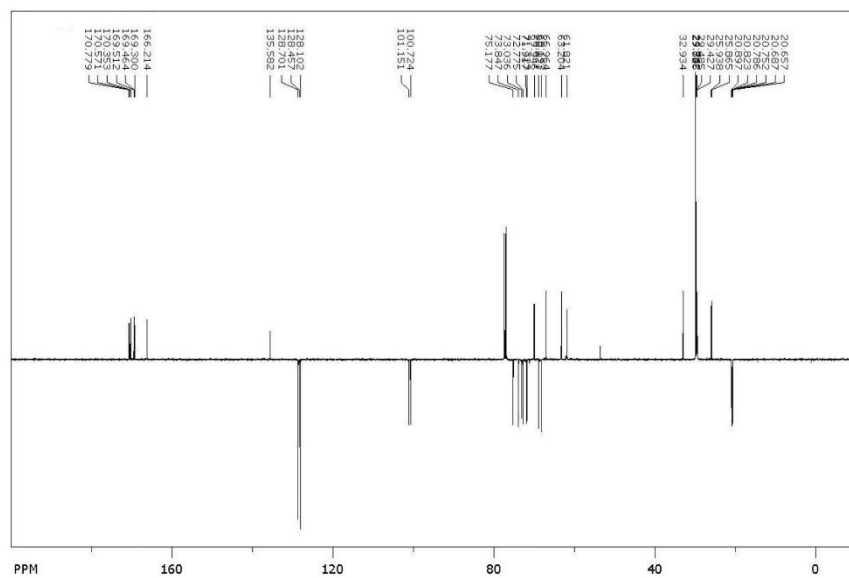
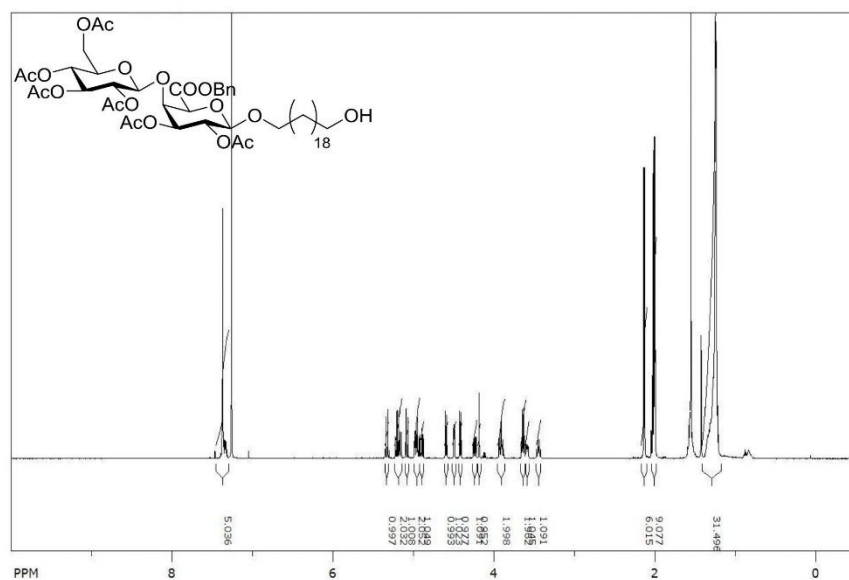


(1-(*tert*-Butyl-dimethyl-silyloxy)-eicosyl)-(2'',3'',4'',6''-tetra-*O*-acetyl-β-D-glucopyranosyl)-(1→4)-2',3'-di-*O*-acetyl-1'-*O*-β-D-galactopyranosiduronic acid (**29**)

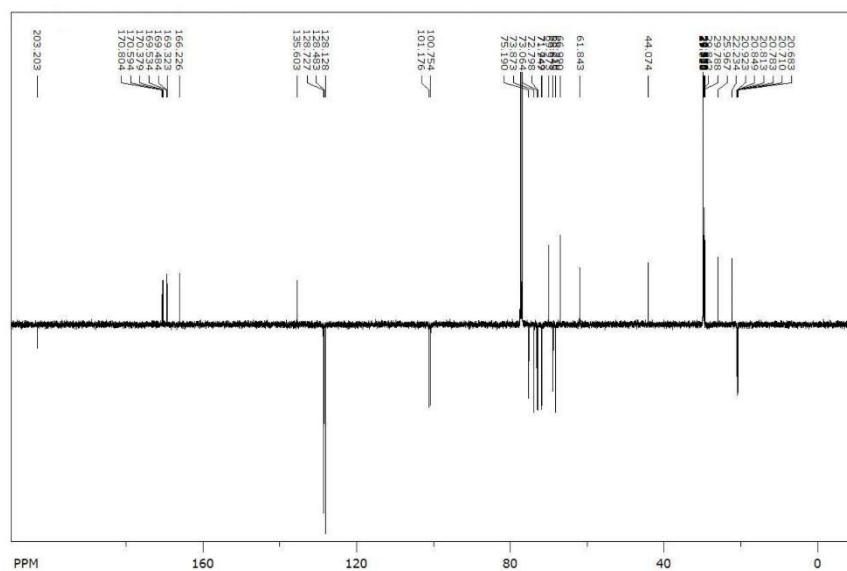
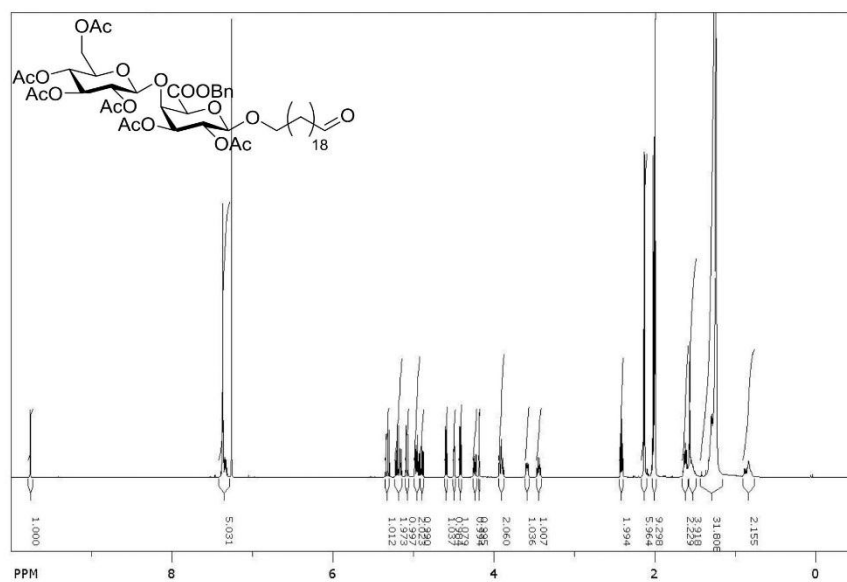


Benzyl-((1-(*tert*-butyl-dimethyl-silyloxy)-eicosyl)-(2'',3'',4'',6'')-tetra-*O*-acetyl- β -D-glucopyranosyl)-(1 \rightarrow 4)-2',3'-di-*O*-acetyl-1'-*O*- β -D-galactopyranoside) uronate (**30**)

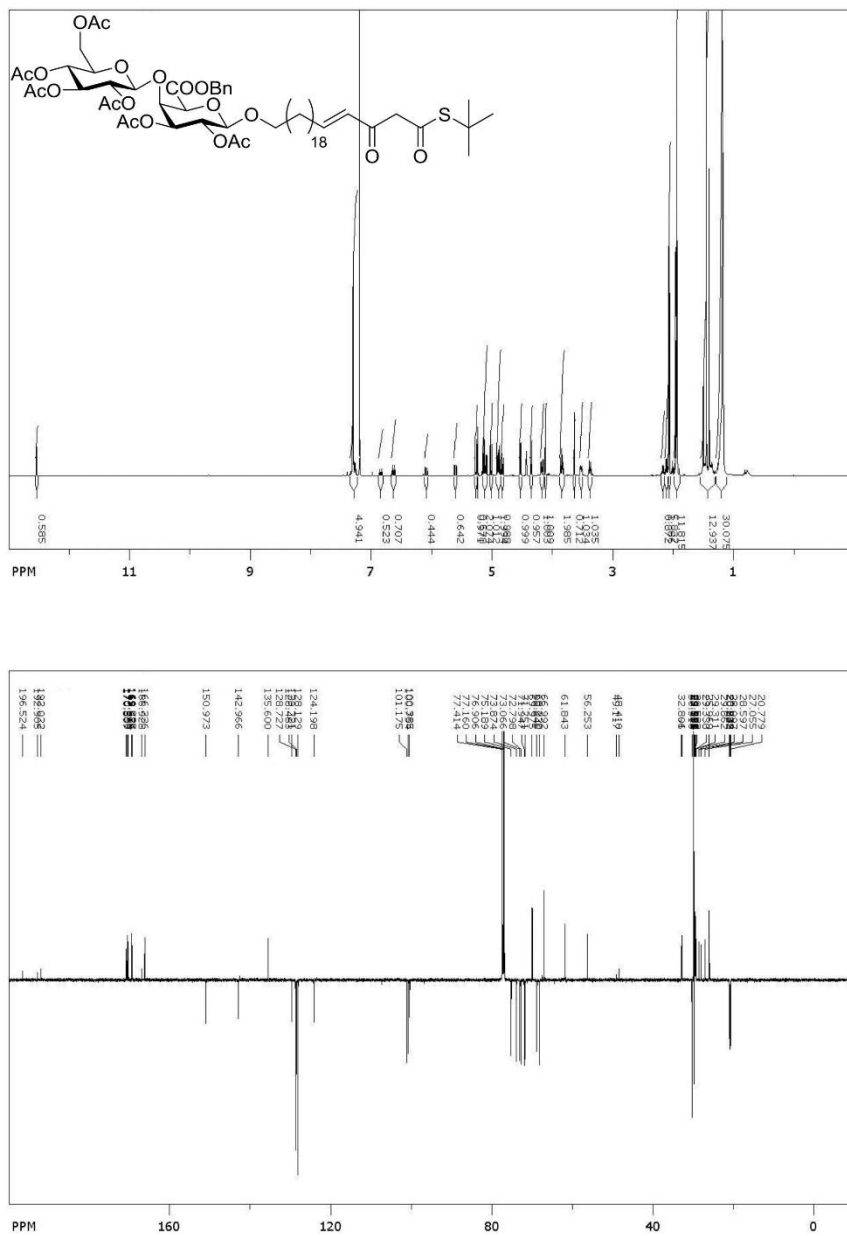




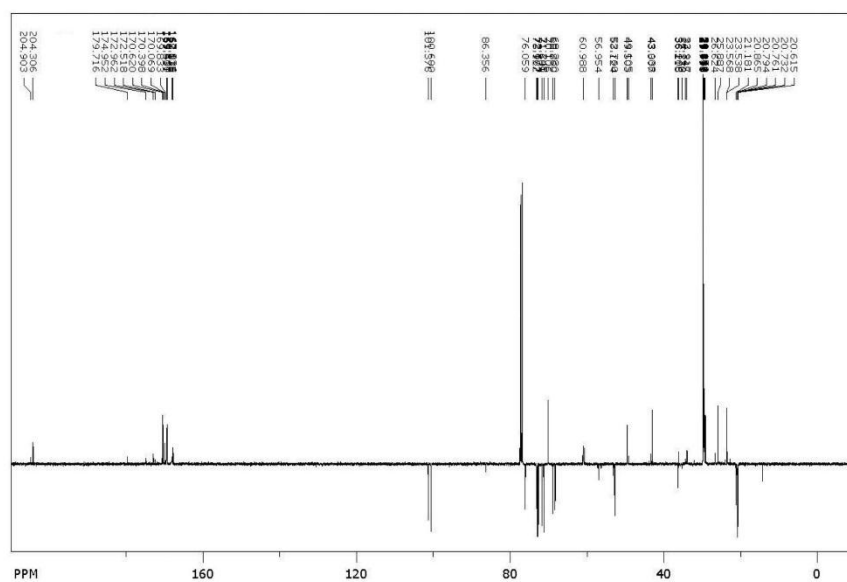
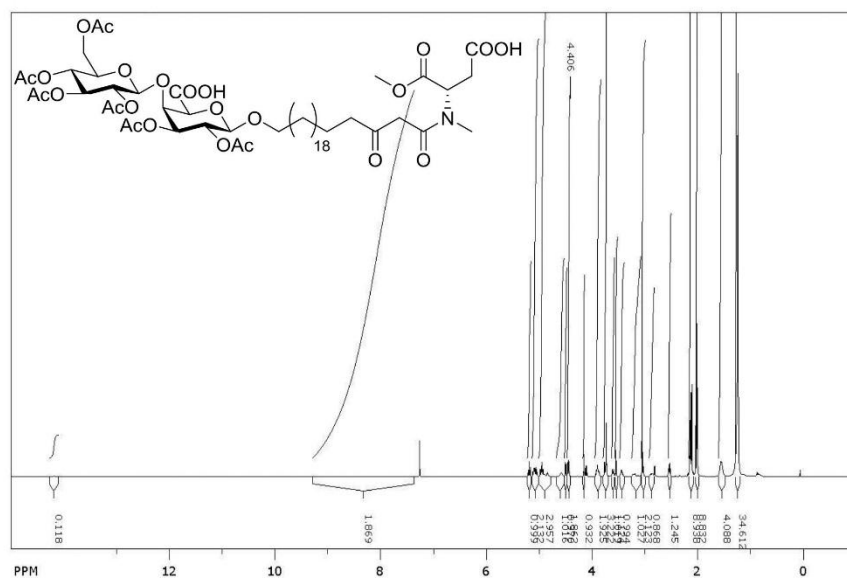
Benzyl-(1-oxo-eicosyl-(2'',3'',4'',6'')-tetra-O-acetyl- β -D-glucopyranosyl)-(1 \rightarrow 4)-2',3'-di-O-acetyl-1'-O- β -D-galactopyranoside) uronate (**8**)



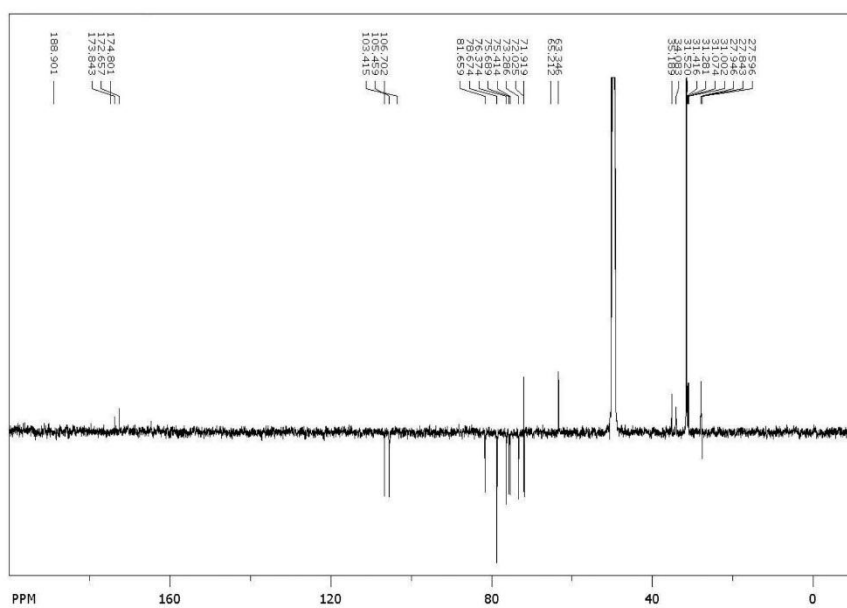
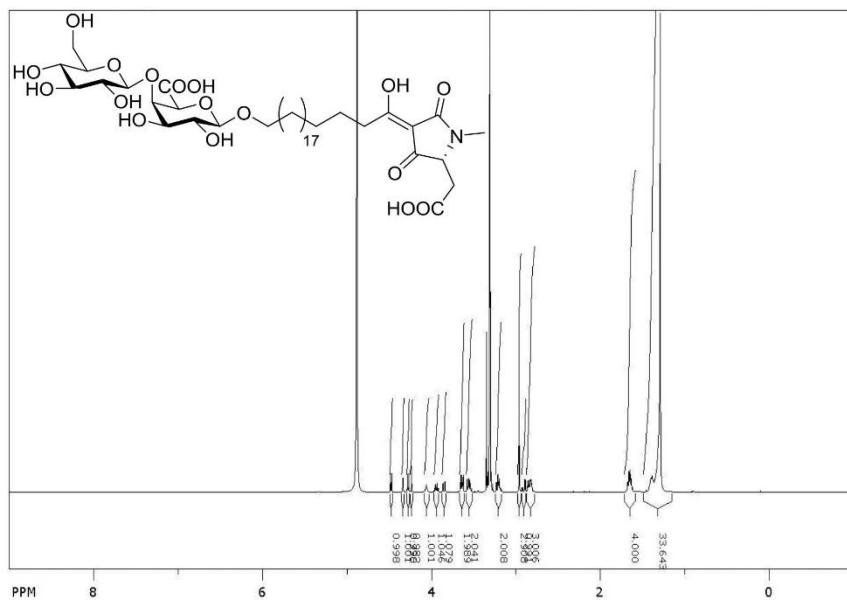
Benzyl-(3-oxo-1-(*S-tert*-butylthiocarbonyl-eicosa-4-enyl)-(2'',3'',4'',6'')-tetra-*O*-acetyl- β -D-glucopyranosyl)-(1 \rightarrow 4)-2',3'-di-*O*-acetyl-1'-*O*- β -D-galactopyranoside uronate (**6**)

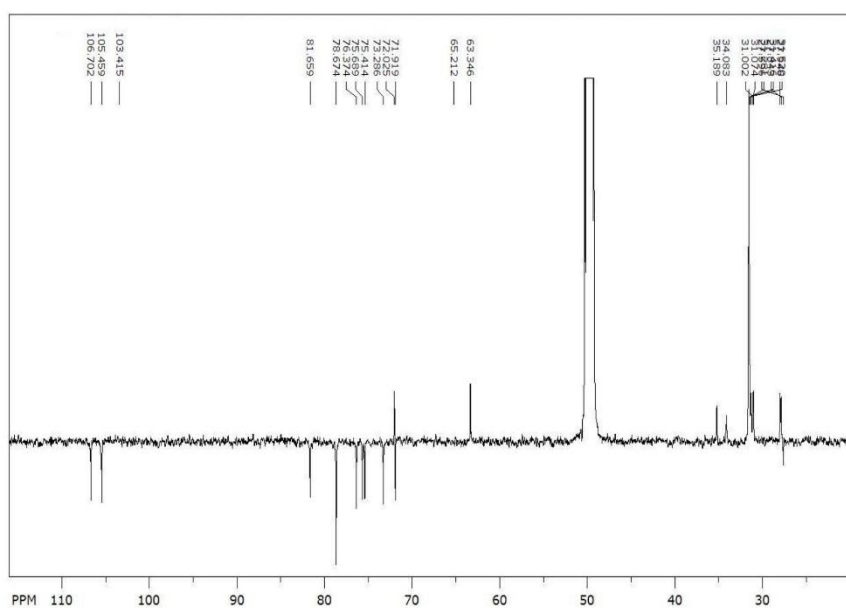
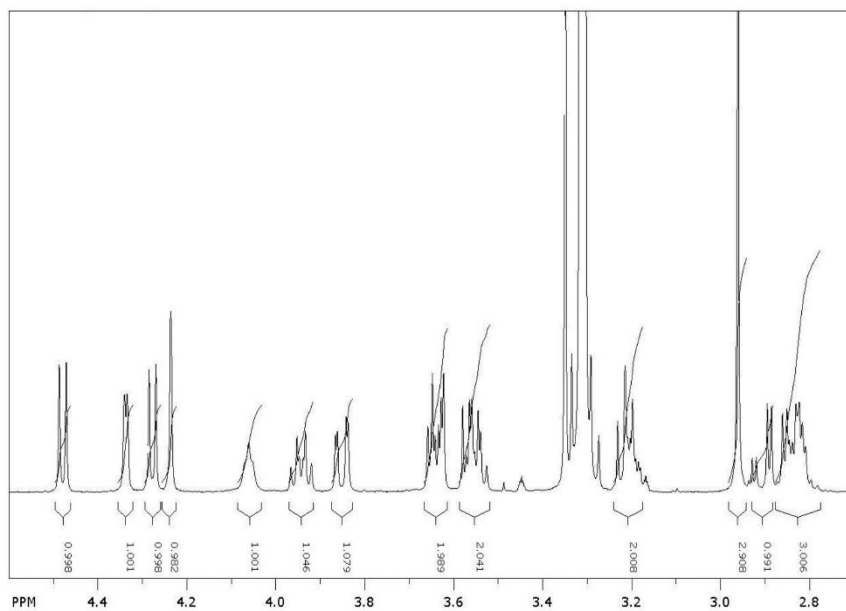


(3*R*)-4-Aza-4-methyl-3-(methoxycarbonyl)-5,7-di-oxo-octacosanic acid)-(2'',3'',4'',6''-tetra-*O*-acetyl-β-D-glucopyranosyl)-(1→4)-2',3'-di-*O*-acetyl-1'-*O*-β-D-galactopyranosiduronic acid (**5**)

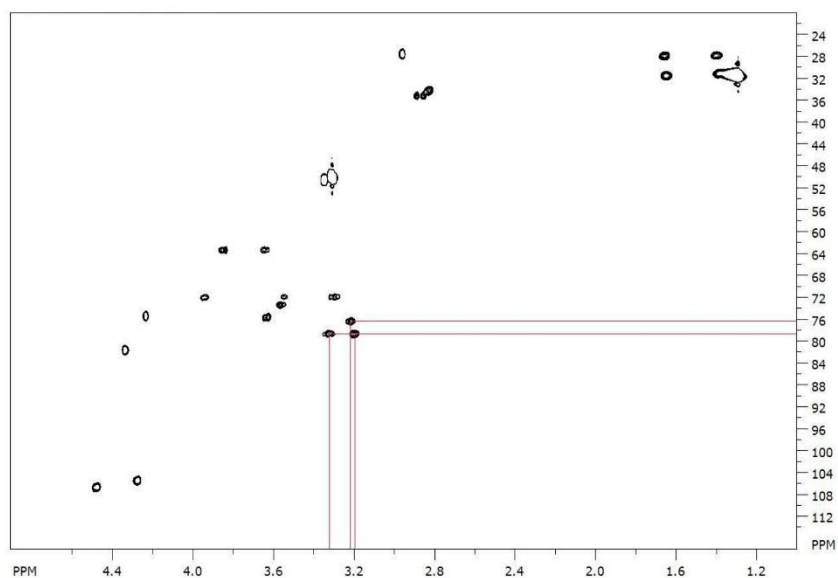


Ancorinoside A (1)

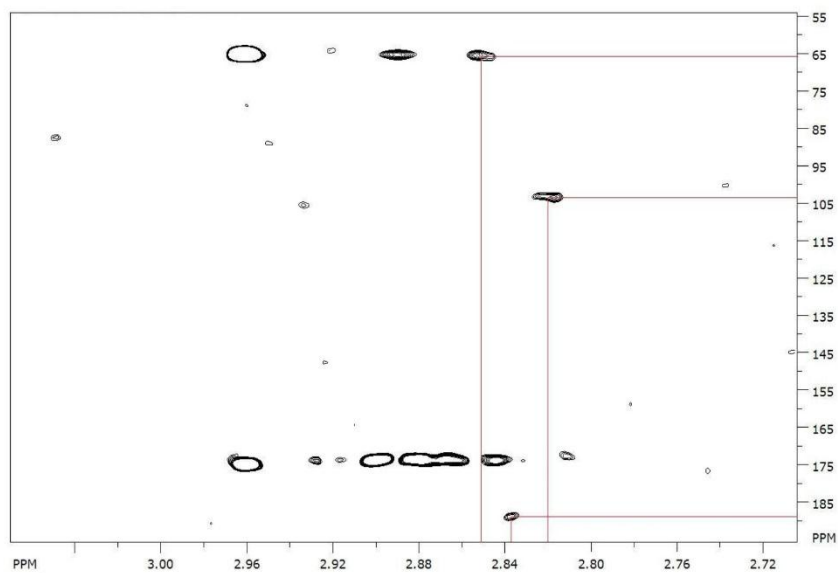




HSQC spectra of ancorinoside A (**1**) for the identification of 3''-H to 5''-H

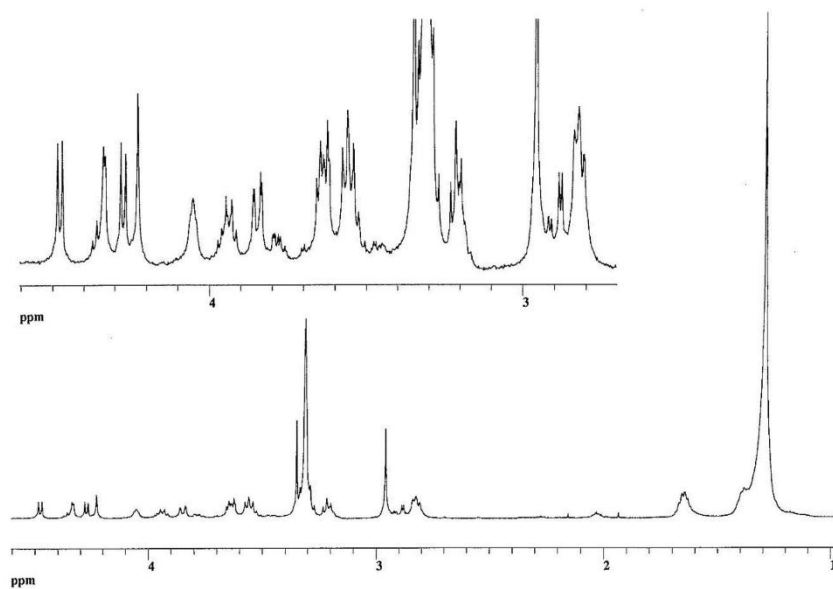


HMBC spectra of ancorinoside A (**1**) for the identification of C2 to C4

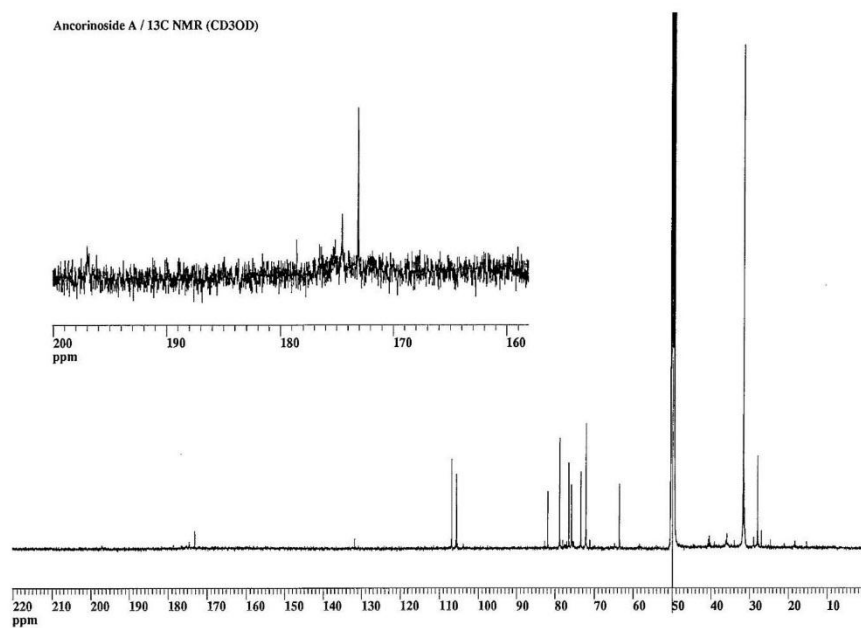


^1H and ^{13}C NMR spectra of isolated ancorinoside A (**1**) Ohta *et al.*^[10]

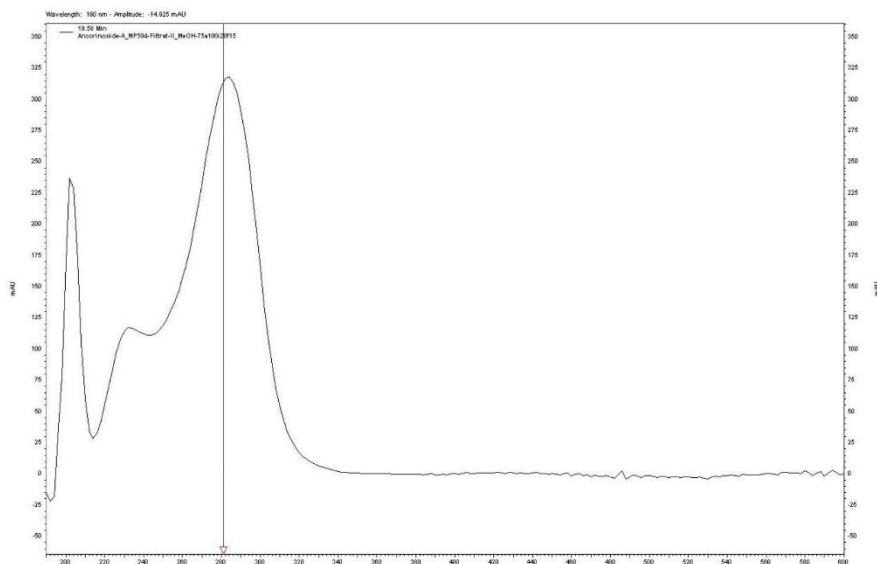
Ancorinoside A / ^1H NMR (CD₃OD)



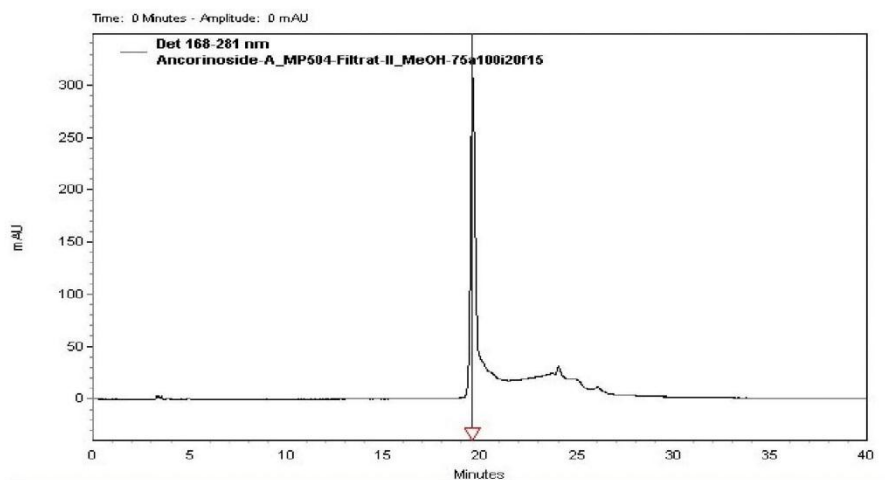
Ancorinoside A / ^{13}C NMR (CD₃OD)



HPLC UV absorption maxima at 19.58 min.



HPLC chromatogram



HPLC program: start at 75% MeOH in H₂O with 0.1% HCOOH to 100% MeOH in 20 min for 10 min;
Flow rate 0.7 mL/min, column: Phenomenex Kinetex 5 μ m C18 100 Å, 250 x 4.60 mm

6.3 Publikation III

A Synthetic Route to the MT1-MMP Inhibitor Ancorinoside D

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95447 Bayreuth, Germany

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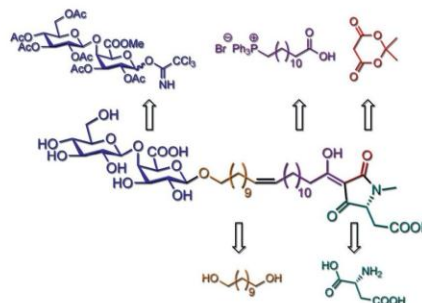
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A Synthetic Route to the MT1-MMP Inhibitor Ancorinoside D

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Abstract A methyl ester of ancorinoside D, a 3-acyltetramic acid metabolite of a sponge *Penares sollasi*, was synthesised in ten steps starting from a protected β -D-glucopyranosyl-(1 \rightarrow 4)-D-galactopyranosyltrichloroacetimidate donor. Its attachment to the left half of the 3-acyl spacer by a Schmidt glycosylation, 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO)-mediated oxidation to the uronic acid, introduction of the Z-alkene via Wittig reaction, and functionalisation of the spacer terminus with Meldrum's acid gave a β -keto ester that reacted with dimethyl N-methyl-D-aspartate under neutral conditions to afford a fully protected ancorinoside D as the product of an unusual domino N-acylation–Dieckmann condensation. Global deprotection left a methyl ester of ancorinoside D, which resisted all saponification attempts.

Key words tetramic acid, Wittig reaction, Dieckmann cyclisation, natural products, glycosylation, ancorinoside, MMP inhibitor, domino reaction

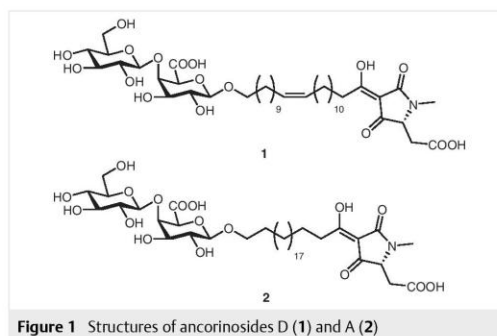


Figure 1 Structures of ancorinosides D (1) and A (2)

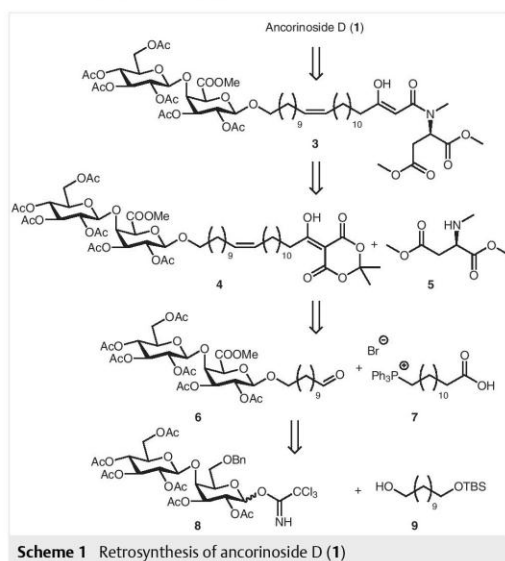
In 2001, the glycosylated tetramic acid ancorinoside D (1; Figure 1) was isolated as its tris(diethylammonium) salt, together with two structurally related derivatives, ancorinoside B and C, from the marine sponge *Penares sollasi* Thiele by Fujita et al. during their search for specific inhibitors of membrane-type matrix metalloproteinases (MT1-MMPs).¹ These Zn-dependent enzymes are interesting pharmacological targets that play a crucial role in cell migration and the transformation of the extracellular matrix, which are processes that are themselves key to wound healing, angiogenesis and inflammation, but also to tumour growth and metastasis.²

Ancorinoside D (1) \times (NHET₂)₃ was found to be a 2.5 times better inhibitor of MT1-MMP when compared to its close congener ancorinoside A (2), which had been isolated in 1997 by Ohta et al.³ from a sponge *Ancorina* sp. and which was synthesised, as the first member of the ancori-

nosiide family, by our group in 2017.⁴ To evaluate the flexibility and applicability of our synthetic approach, and for a detailed investigation of the structure dependency of the MT1-MMP inhibition effect, we now wanted to also prepare tetramic acid 1.

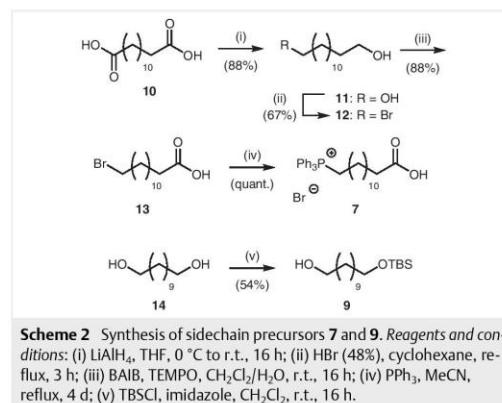
As in our synthesis of ancorinoside A (2),⁴ we used the protected disaccharide trichloroacetimidate donor 8 and a monosilylated derivative of an appropriate α,ω -diol, in this case the TBS-ether 9 of undecane-1,11-diol (14), to build up the left half of the 3-acyl sidechain of ancorinoside D (1) (Scheme 1). After protection group modification and oxidation of the disaccharide to the uronic acid, the undecyl appendage was deprotected and oxidised to the corresponding aldehyde 6. The remaining half of the sidechain and the Z-alkene were introduced via Wittig reaction between aldehyde 6 and the ylide derived from ω -(triphenylphosphonium)tridecanoic acid bromide (7). Due to the presence of the Z-alkene, we could not, as we did in the synthesis of the saturated 3-acyl sidechain of 2, use a β -keto thioester head group to N-acylate N-methyl-D-aspartate, the precursor of the tetramic acid fragment of 1 and 2. Such a β -keto thio-

ester would have to be introduced by a HWE olefination⁵ followed by hydrogenation of the so created alkene, which was not possible to do selectively in the presence of a second alkene. Therefore, we condensed the carboxylic acid, obtained from the Wittig reaction, with Meldrum's acid and used the resulting β -keto ester **4** to *N*-acylate dimethyl *N*-methyl-D-aspartate (**5**). The product β -ketoamide **3** was expected to undergo a Dieckmann cyclisation to give the tetracarboxylic acid under the basic conditions required for removal of the acetyl protecting groups.



Phosphonium salt **7** was synthesised in four steps and 52% yield starting from commercially available undecane-1,11-dicarboxylic acid (**10**; Scheme 2). Its reduction with LiAlH_4 afforded diol **11**, which was converted into ω -bromotridecanol (**12**).⁶ Reoxidation with BAIB/TEMPO to the corresponding ω -bromocarboxylic acid **13**, followed by nucleophilic substitution of the bromide with PPh_3 , afforded phosphonium salt **7**.^{7,8} The glycosyl acceptor **9** was synthesised by a monosilylation of undecane-1,11-diol (**14**) with

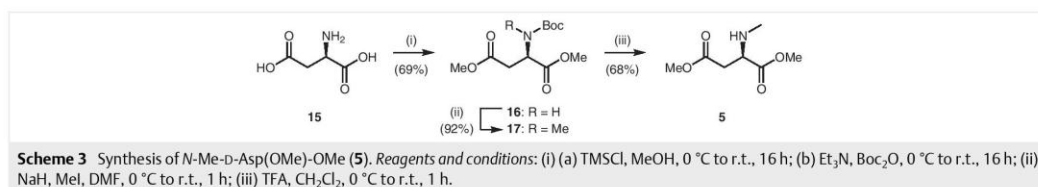
imidazole as a base in an expected yield of ca. 50%.⁹ The usual monosilylation protocol¹⁰ for symmetric diols using NaH gave only a mixture of mono- and disilylated products.



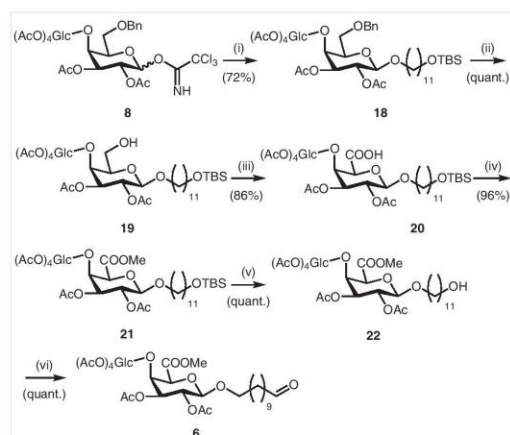
The required *N*-methyl-D-aspartate was prepared as described in our synthesis of ancorinoside A (**2**).⁴ Due to the alkene in the spacer of **1**, we used the methyl ester **5** rather than the benzyl ester.

Inexpensive D-aspartic acid (**15**) was converted in situ into its dimethylester hydrochloride with a mixture of TMSCl and methanol. Addition of Boc_2O and Et_3N to this mixture furnished dimethyl *N*-Boc-D-aspartate (**16**; Scheme 3).¹¹ The latter was deprotonated with NaH and methylated with MeI to give the *N*-methyl-*N*-Boc intermediate **17** as a mixture of rotamers, typical of tertiary carbamates.¹² It was deprotected by TFA, as swiftly as possible, to prevent the formation of the corresponding *N*-methyl-*N*-carboxyanhydride.¹³ The product, dimethyl *N*-methyl-D-aspartate (**5**), was obtained in 43% over three steps.

The fourth building block, disaccharide donor **8**, was prepared in eight steps from D-galactose and D-glucose via a thioglycoside glycosylation as previously described for the synthesis of ancorinoside A.⁴ The assembly of the target molecule **1** began with the glycosylation of acceptor **9** with donor **8** (Scheme 4). The α,β -isomeric mixture of donor **8** was connected β -selectively with 11-(*tert*-butyldimethylsilyloxy)undecan-1-ol (**9**) according to a protocol by Schmidt et al.¹⁴ A repeatedly observed cleavage of the acetyl group at the glucose C-2 during this reaction could eventually be



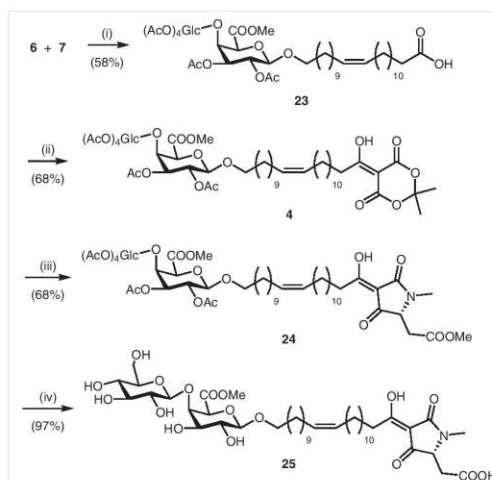
suppressed by a prolonged reaction time and a faster warm-up. Quantitative hydrogenolytic debenzoylation of product disaccharide **18** left primary alcohol **19**, which was oxidised to the corresponding galacturonic acid **20**. The latter was converted to methyl ester **21** in almost quantitative yield. Acidic cleavage of the terminal silyl group and DMP mediated oxidation of alcohol **22** afforded the key aldehyde **6**. Its purification via column chromatography resulted in a loss of around 40% yield for unknown reasons. An alternative sulfonium ion mediated Parikh Doering oxidation of **22** did not lead to aldehyde **6** in better yield or purity, either.



Scheme 4 Synthesis of aldehyde **6**. Reagents and conditions: (i) TMSOTf, **9**, MS 4Å, CH₂Cl₂, –40 °C to 0 °C, 2.5 h; (ii) 5% Pd/C, H₂ (1 bar), EtOAc, r.t., 2.5 h; (iii) BAIB, TEMPO, CH₂Cl₂/H₂O, r.t., 1 h; (iv) K₂CO₃, MeI, DMF, 0 °C to r.t., 2.5 h; (v) AcOH/THF/H₂O, r.t., 2 h; (vi) DMP, CH₂Cl₂, r.t., 1 h.

In a first attempt to introduce the alkene, we reacted aldehyde **6** with the ylide generated from (13-*tert*-butyldimethylsilyloxy)tridecyltriphenylphosphonium bromide, which was obtained from bromoalcohol **12**. Subsequent desilylation and TEMPO mediated oxidation afforded enoic acid **23** in poor yield. However, a one-step Wittig olefination with the ylide derived from phosphonium salt **7** gave a better yield of enoic acid **23** when NaHMDS was used as a base (Scheme 5). In agreement with previous reports using the same ylide and similar aldehydes, we only found the *Z*-diastereomer.^{8a,15} Acid **23** was condensed with Meldrum's acid to give the β-keto ester **4**,^{16a} required for the *N*-acylation of aspartate **5**.

Deviating from reported protocols¹⁷ for such acylation reactions, Meldrum's acid derivative **4** could not be used without prior purification, which was best done by rapid column chromatography over a short plug of silica to avoid



Scheme 5 Synthesis of methyl ester **25**. Reagents and conditions: (i) NaHMDS, THF, –78 °C to r.t., 2 h; (ii) DMAP, EDCI, Meldrum's acid, CH₂Cl₂, 0 °C to r.t., 16 h; (iii) **5**, toluene, reflux, 4 h; (iv) NaOH, CH₂Cl₂/MeOH, r.t., 30 min.

decomposition due to the acidic character of the silica.¹⁷ Gratifyingly, the aminolysis of purified **4** with *N*-Me-D-Asp(OMe)-OMe (**5**), when carried out in refluxing toluene, gave none of the expected β-ketoamide **3**¹⁸ but immediately its Dieckmann condensation product, the 3-acyltetramic acid **24**. In previous syntheses of natural 3-acyltetramic acids, we found that reactions of acyl derivatives of Meldrum's acid with aminoesters afforded isolable *N*-(β-ketoacyl)aminoesters while their cyclisation to the corresponding tetramic acids required the addition of base.^{16b,19}

As the final step in our synthesis of ancorinoside D (**1**), we intended to globally deprotect the oligo-ester **24** under carefully adjusted basic conditions. The use of aqueous LiOH, often applied to cleave ester groups in similar functional group constellations, left one methyl ester and also gave rise to some unidentified inseparable by-product after only 1 h reaction time.²⁰ Although NMR studies were inconclusive as to which of the two esters evaded saponification, we assume the methyl galacturonate remains intact because of its steric encumbrance. Saponification of **24** with milder non-aqueous NaOH also afforded ester **25**, advantageously free of the unknown by-product as long as an excess of base was avoided.²¹ Attempts at cleaving all esters of **24** by means of enzymes also failed. Neither pig liver esterase, an enzyme widely used to this end, nor lipase from porcine pancreas type II, often employed for selective acetyl cleavage, gave any conversion, even when the reaction time was extended to two weeks and with organic co-solvents added.

We are currently testing alternative methods, e.g., S_N2 reactions and reductive ester cleavage protocols, to liberate the target molecule **1**.²² We will also try out different, more readily cleavable esters, such as trimethylsilylethyl (TMSE), to protect galacturonic acid **20**. The results of these studies and of MMP inhibition assays with ester **25** and **1** will be reported in a separate communication.

In conclusion, we demonstrated the applicability of our synthetic approach to ancorinoside A (**2**) to another member of this pharmaceutically interesting family of glycosylated tetramic acids, differing in the length and (un)saturation of the sidechain spacer. A few mechanistic abnormalities in our modified synthesis of ancorinoside D (**1**) warrant more detailed investigations, in particular the spontaneous Dieckmann cyclisation of the intermediate *N*-(β -ketoacyl)aminoester under neutral conditions, as well as the problematic saponification of a methyl galacturonate as part of a diglycoside. The purified ancorinoside D methyl ester **25** will be tested for inhibition of a broader series of Zn-dependent matrix metalloproteinases.

IR spectra were recorded with an FTIR spectrophotometer with ATR sample unit. ^1H and ^{13}C NMR spectra were obtained with a Bruker DRX 500 spectrometer. Chemical shifts are given in parts per million using the residual solvent peak as an internal standard according to Fulmer et al.: $\delta = 7.26$ ppm (proton) and 77.16 ppm (carbon) for CDCl_3 and $\delta = 3.31$ ppm (proton) and 49.00 ppm (carbon) for CD_3OD .²³ Coupling constants (*J*) are quoted in Hertz. Multiplicity abbreviations are: s singlet, d doublet, t triplet, q quartet, quin quintet and m multiplet. Mass spectra were measured with a Varian MAT 8500 (EI, 70 eV). High-resolution mass spectra were obtained with an UPLC/Orbitrap MS system in ESI mode. Specific optical rotations were measured with a Perkin-Elmer polarimeter 241 at 589 nm (Na-D-line) and are quoted in $10^\circ \text{ dm}^{-1} \text{ mL mg}^{-1}$. Melting points were taken with an Electrothermal 9100 apparatus and are uncorrected. For chromatography, silica gel 40–63 μm (230–400 mesh) was used. All reagents were purchased from commercial sources and were used without further purification. All reactions were carried out under an argon atmosphere. All anhydrous solvents were freshly distilled according to standard protocols and stored under an argon atmosphere. Analytical thin-layer chromatography was carried out using Merck TLC silica gel 60 F_{254} aluminium sheets. The disaccharide trichloroacetimidate donor **8** was synthesised according to previous reports.⁴

11-(*tert*-Butyldimethylsilyloxy)undecan-1-ol (**9**)

A solution of undecane-1,11-diol (**14**; 1.883 g, 10.00 mmol, 1.00 equiv) in CH_2Cl_2 (50 mL) at r.t. was treated with TBSCl (1.658 g, 11.00 mmol, 1.10 equiv) and imidazole (1.021 g, 15.00 mmol, 1.50 equiv). The suspension was stirred for 16 h at this temperature, water (25 mL) was added, and the mixture was extracted with CH_2Cl_2 (3 \times 25 mL). The combined organic phases were washed with water, dried over anhydrous Na_2SO_4 and the solvent was evaporated under reduced pressure. The remaining residue was purified by column chromatography (cyclohexane/EtOAc, 9:1) to afford the title compound. Yield: 1.641 g (5.424 mmol, 54%); colourless oil; $R_f = 0.55$ (cyclohexane/EtOAc, 3:1).

IR (ATR): 3362, 2926, 2855, 1470, 1463, 1388, 1361, 1254, 1098, 1056, 1006, 939, 833, 815, 773, 721, 661 cm^{-1} .

^1H NMR (CDCl_3 , 500 MHz): $\delta = 3.63$ (dt, *J* = 5.5, 6.5 Hz, 2 H, 1-H), 3.59 (t, *J* = 6.7 Hz, 2 H, 11-H), 1.60–1.47 (m, 4 H, 2-H, 10-H), 1.38–1.21 (m, 15 H, 3-H to 9-H, OH), 0.89 (s, 9 H, *t*Bu), 0.04 (s, 6 H, Me).

^{13}C NMR (CDCl_3 , 125 MHz): $\delta = 63.5$, 63.3, 33.03, 32.96, 29.76, 29.74, 29.67, 29.58, 29.57, 26.1, 25.94, 25.88, 18.5, –5.1.

HRMS (ESI): *m/z* [*M* + *H*]⁺ calcd for $\text{C}_{17}\text{H}_{39}\text{O}_2\text{Si}$: 303.27138; found: 303.27159.

Tridecane-1,13-diol (**11**)^{6a}

To a suspension of LiAlH_4 (0.835 g, 22.00 mmol, 2.20 equiv) in THF (50 mL) was slowly added a solution of undecane-1,11-dioic acid (**10**; 2.443 g, 10.00 mmol, 1.00 equiv) in THF (100 mL) at 0 $^\circ\text{C}$. This mixture was stirred at r.t. for 16 h. The reaction was stopped by addition of a 30 wt% aqueous solution of citric acid at 0 $^\circ\text{C}$ (100 mL). The mixture was extracted with Et_2O (3 \times 80 mL) and the combined organic phases were washed with H_2O , dried over anhydrous Na_2SO_4 and the solvent was evaporated under reduced pressure. The residue was dissolved in Et_2O and washed again with H_2O . The organic phase was dried over anhydrous Na_2SO_4 and the solvent was evaporated under reduced pressure. The product was used without further purification.

Yield: 1.905 g (8.804 mmol, 88%); colourless solid; mp 74–75 $^\circ\text{C}$ (lit.^{6b} mp 75–76 $^\circ\text{C}$); $R_f = 0.39$ (cyclohexane/EtOAc, 2:3).

IR (ATR): 3236, 2917, 2849, 1486, 1472, 1462, 1405, 1357, 1344, 1324, 1302, 1257, 1210, 1187, 1123, 1057, 1037, 1019, 1002, 973, 958, 920, 876, 831, 764, 731, 720, 687 cm^{-1} .

^1H NMR (CDCl_3 , 500 MHz): $\delta = 3.65$ (t, *J* = 6.6 Hz, 4 H, 1-H, 13-H), 1.57 (quin, *J* = 6.6 Hz, 4 H, 2-H, 12-H), 1.38–1.25 (m, 20 H, 3-H to 11-H, OH).

^{13}C NMR (CDCl_3 , 125 MHz): $\delta = 63.3$, 33.0, 29.74, 29.73, 29.70, 29.6, 25.9.

13-Bromotridecan-1-ol (**12**)^{6a}

Aqueous HBr (48%, 16 mL) was added under vigorous stirring to diol **11** (2.644 g, 12.22 mmol, 1.00 equiv) suspended in cyclohexane (32 mL). The mixture was stirred and heated to reflux for 3 h, and the aqueous phase was separated after cooling to r.t. and extracted with EtOAc (3 \times 30 mL). The combined organic phases were washed with brine, dried over anhydrous Na_2SO_4 and the solvent was evaporated under reduced pressure. The remainder was purified by column chromatography (cyclohexane/EtOAc, 3:1) to afford the title compound.

Yield: 2.284 g (8.179 mmol, 67%); colourless solid; mp 54–55 $^\circ\text{C}$ (lit.^{6b} mp 56–57 $^\circ\text{C}$); $R_f = 0.51$ (cyclohexane/EtOAc, 3:1).

IR (ATR): 3428, 2917, 2851, 1468, 1355, 1327, 1253, 1205, 1058, 1049, 1025, 1010, 995, 969, 722, 645, 604, 570 cm^{-1} .

^1H NMR (CDCl_3 , 500 MHz): $\delta = 3.64$ (dt, *J* = 5.5, 6.5 Hz, 2 H, 1-H), 3.41 (t, *J* = 7.0 Hz, 2 H, 13-H), 1.85 (quin, *J* = 7.0 Hz, 2 H, 12-H), 1.57 (quin, *J* = 6.5 Hz, 2 H, 2-H), 1.46–1.24 (m, 18 H, 3-H to 11-H), 1.19 (t, *J* = 5.5 Hz, 1 H, OH).

^{13}C NMR (CDCl_3 , 125 MHz): $\delta = 63.3$, 34.3, 32.98, 32.96, 29.74, 29.70, 29.67, 29.57, 28.92, 28.33, 25.9.

13-Bromotridecanoic Acid (**13**)

An emulsion of alcohol **12** (1.910 g, 6.840 mmol, 1.00 equiv) in CH_2Cl_2 (44 mL) and H_2O (22 mL) was treated with BAIB (4.847 g, 15.05 mmol, 2.20 equiv) and TEMPO (0.214 g, 1.37 mmol, 0.20 equiv) at r.t. The mixture was stirred at this temperature for 16 h, an aqueous $\text{Na}_2\text{S}_2\text{O}_3$

solution (10 wt%, 50 mL) was added, the phases were separated, and the aqueous phase was extracted with EtOAc (3 × 50 mL). The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. The residue was purified by column chromatography (cyclohexane/EtOAc, 9:1 + 0.1% HCOOH) to afford the title compound.

Yield: 1.768 g (6.029 mmol, 88%); colourless solid; mp 54–56 °C (lit.²⁴ mp 53 °C); *R*_f = 0.23 (cyclohexane/EtOAc, 4:1).

IR (ATR): 3009, 2916, 2849, 2646, 1699, 1472, 1463, 1431, 1411, 1354, 1316, 1296, 1272, 1246, 1223, 1202, 1185, 939, 910, 729, 721, 650 cm⁻¹.

¹H NMR (CDCl₃, 500 MHz): δ = 11.29 (br s, 1 H, COOH), 3.41 (t, *J* = 6.9 Hz, 2 H, 13-H), 2.35 (t, *J* = 7.6 Hz, 2 H, 2-H), 1.85 (quin, *J* = 6.9 Hz, 2 H, 12-H), 1.63 (quin, *J* = 7.6 Hz, 2 H, 3-H), 1.45–1.39 (m, 2 H, 4-H), 1.35–1.23 (m, 14 H, 5-H to 11-H).

¹³C NMR (CDCl₃, 125 MHz): δ = 179.9, 34.2, 34.1, 33.0, 29.63, 29.61, 29.53, 29.51, 29.3, 29.2, 28.9, 28.3, 24.8.

12-Carboxydodecyltriphenylphosphonium Bromide (7)⁸

A suspension of **13** (1.064 g, 3.625 mmol, 1.00 equiv) and PPh₃ (0.960 g, 3.625 mmol, 1.00 equiv) in MeCN (3.6 mL) was heated at 90 °C for 4 d. After cooling to r.t., the solvent was evaporated under reduced pressure. The remainder was dissolved in a small amount of CHCl₃ and precipitated with Et₂O to afford the title compound.

Yield: 2.015 g (3.625 mmol, quant.); colourless solid; mp 85–87 °C.

IR (ATR): 2922, 2851, 1715, 1587, 1482, 1467, 1436, 1398, 1379, 1337, 1315, 1211, 1178, 1162, 1111, 1073, 1025, 995, 931, 854, 790, 748, 723, 690, 614 cm⁻¹.

¹H NMR (CDCl₃, 500 MHz): δ = 7.85–7.74 (m, 9 H, CH-Ph), 7.73–7.66 (m, 6 H, CH-Ph), 3.75–3.59 (m, 2 H, 13-H), 2.35 (t, *J* = 7.4 Hz, 2 H, 2-H), 1.67–1.50 (m, 6 H, 3-H, 4-H, 12-H), 1.27–1.15 (m, 14 H, 5-H to 11-H).

¹³C NMR (CDCl₃, 125 MHz): δ = 177.8, 135.2, 135.1, 133.8, 133.7, 130.7, 130.6, 118.8, 118.1, 34.4, 30.5, 30.4, 29.2, 29.1, 29.0, 28.98, 28.87, 24.8, 23.0, 22.72, 22.68, 22.57.

D-N-Boc-Asp(OMe)-OMe (16)¹¹

A suspension of D-aspartic acid (**15**; 1.331 g, 10.00 mmol, 1.00 equiv) in MeOH (27 mL) was treated dropwise with TMSCl (5.5 mL, 44.00 mmol, 4.40 equiv) at 0 °C. The reaction mixture was warmed to r.t. after 1 h and stirred at this temperature for 16 h. The mixture was cooled again to 0 °C and treated with Et₃N (9 mL, 65.00 mmol, 6.50 equiv) and Boc₂O (2.401 g, 11.00 mmol, 1.10 equiv). The reaction was warmed to r.t., stirred for a further 16 h at this temperature, and diluted with EtOAc and H₂O. The aqueous phase was extracted with EtOAc (3 × 50 mL), and the combined organic phases were washed with brine, dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. The residue was purified by column chromatography (cyclohexane/EtOAc, 4:1) to afford the title compound.

Yield: 1.802 g (6.897 mmol, 69%); colourless solid; mp 64–65 °C (lit.^{11b} isomer mp 58–60 °C); [α]_D²⁵ –32.7 (lit.^{11b} isomer +30.8) (*c* = 1.00 CHCl₃); *R*_f = 0.65 (cyclohexane/EtOAc, 2:1).

IR (ATR): 3380, 2979, 1738, 1716, 1503, 1438, 1393, 1367, 1347, 1290, 1215, 1163, 1047, 1028, 998, 847, 811, 781 cm⁻¹.

¹H NMR (CDCl₃, 500 MHz): δ = 5.49 (d, *J* = 8.4 Hz, 1 H, NH), 4.58 (dt, *J* = 4.5, 8.4 Hz, 1 H, 2-H), 3.76 (s, 3 H, OMe), 3.70 (s, 3 H, OMe), 3.01 (dd, *J* = 4.5, 17.2 Hz, 1 H, 3-H^a), 2.83 (dd, *J* = 4.6, 17.2 Hz, 1 H, 3-H^b), 1.45 (s, 9 H, tBu).

¹³C NMR (CDCl₃, 125 MHz): δ = 171.7, 171.6, 155.5, 80.3, 52.9, 52.2, 50.0, 36.8, 28.4.

MS (EI): *m/z* (%) = 41 (71) [C₂H₅O]⁺, 57 (100) [C₄H₉]⁺, 76 (31), 86 (43), 102 (99), 113 (26), 128 (42), 146 (99), 160 (65) [C₆H₁₀NO₄]⁺, 174 (39), 188 (20), 202 (82) [C₉H₁₆NO₄]⁺.

D-N-Me-N-Boc-Asp(OMe)-OMe (17)

A suspension of NaH (60% in mineral oil, 0.048 g, 1.20 mmol, 1.20 equiv) in DMF (4 mL) was slowly treated with a solution of methyl iodide (0.249 mL, 4.00 mmol, 4.00 equiv) and **16** (0.261 g, 1.00 mmol, 1.00 equiv) in DMF (8 mL) at 0 °C. The mixture was warmed to r.t., stirred for 1 h, diluted with a saturated aqueous NH₄Cl solution, and extracted with EtOAc (3 × 20 mL). The combined organic phases were washed with H₂O and brine, dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. The remainder was purified by column chromatography (cyclohexane/EtOAc, 3:1) to afford **17**. Because of its instability, the title product was immediately used for the next reaction step.

Yield: 0.254 g (0.923 mmol, 92%); colourless oil; *R*_f = 0.48 (cyclohexane/EtOAc, 2:1).

IR (ATR): 2978, 2952, 1742, 1702, 1483, 1439, 1394, 1368, 1283, 1257, 1230, 1152, 1007, 869, 775, 622, 608, 583, 561 cm⁻¹.

¹H NMR (CDCl₃, 500 MHz): δ = 4.82–4.47 (m, 1 H, 2-H), 3.79–3.67 (m, 6 H, OMe), 3.14–3.06 (m, 1 H, 3-H^a), 2.97–2.87 (m, 3 H, NMe), 2.82–2.72 (m, 1 H, 3-H^b), 1.47–1.41 (m, 9 H, tBu).

¹³C NMR (CDCl₃, 125 MHz): δ = 171.6, 171.2, 81.0, 80.6, 58.3, 57.2, 52.6, 52.2, 52.1, 35.5, 35.2, 34.7, 34.1, 28.4.

HRMS (ESI): *m/z* [M + Na]⁺ calcd for C₁₂H₂₁O₆NNa: 298.12611; found: 298.12582.

D-N-Me-Asp(OMe)-OMe (5)

A solution of **17** (0.253 g, 0.919 mmol, 1.00 equiv) in CH₂Cl₂ (9 mL) was slowly treated with TFA (1.40 mL, 1.84 mmol, 20.00 equiv) at 0 °C. The mixture was warmed to r.t., stirred for 1 h, diluted with EtOAc, and the reaction was quenched by addition of saturated aqueous NaHCO₃ after consumption of the starting material. The aqueous phase was extracted with EtOAc (3 × 20 mL), the combined organic phases were washed with brine, dried over anhydrous Na₂SO₄ and concentrated under reduced pressure to leave pure title compound **5**.

Yield: 0.110 g (0.628 mmol, 68%); colourless oil; [α]_D²⁵ +4.9 (*c* = 1.00 CHCl₃); *R*_f = 0.06 (cyclohexane/EtOAc, 2:1).

IR (ATR): 2955, 2802, 1735, 1483, 1437, 1366, 1263, 1197, 1172, 1127, 1026, 997, 782 cm⁻¹.

¹H NMR (CDCl₃, 500 MHz): δ = 3.76 (s, 3 H, OMe), 3.70 (s, 3 H, OMe), 3.60 (t, *J* = 6.4 Hz, 1 H, 2-H), 2.76 (dd, *J* = 5.7, 16.0 Hz, 1 H, 3-H^a), 2.67 (dd, *J* = 7.0, 16.0 Hz, 1 H, 3-H^b), 2.43 (s, 3 H, NMe), 2.10 (br s, 1 H, NH).

¹³C NMR (CDCl₃, 125 MHz): δ = 173.9, 171.5, 59.4, 52.3, 52.1, 37.3, 34.8.

HRMS (ESI): *m/z* [M + H]⁺ calcd for C₇H₁₄O₄N: 176.09173; found: 176.09189.

(11-(tert-Butyldimethylsilyloxy)undecyl)-2",3",4",6"-tetra-O-acetyl-β-D-glucopyranosyl-(1→4)-2',3'-di-O-acetyl-6'-O-benzyl-1'-O-β-D-galactopyranoside (18)

A mixture of **8** (0.268 g, 0.323 mmol, 1.00 equiv), **9** (0.147 g, 0.485 mmol, 1.50 equiv) and freshly activated molecular sieves 4Å (0.415 g) in CH₂Cl₂ (11 mL) was stirred at r.t. for 1 h before cooling to –40 °C. TMSOTf (5.8 μL, 32.3 μmol, 0.10 equiv) was added to the cooled sus-

pension and the resulting mixture was slowly warmed to 0 °C over 2.5 h. Upon completion of the reaction, the mixture was filtered through Celite, the filtrate was washed with saturated aqueous NaHCO₃ solution, and the aqueous phase was extracted with CH₂Cl₂ (3 × 20 mL). The combined organic phases were washed with water, dried over anhydrous Na₂SO₄, and concentrated under reduced pressure. The remainder was purified by column chromatography (cyclohexane/EtOAc, 7:3) to afford title compound **18**.

Yield: 0.226 g (0.233 mmol, 72%); colourless waxy solid; mp 93–94 °C, $[\alpha]_D^{25}$ –7.0 (*c* = 1.00 CHCl₃); *R*_f = 0.50 (cyclohexane/EtOAc, 1:1). IR (ATR): 2928, 2856, 1750, 1454, 1432, 1367, 1220, 1170, 1096, 1041, 907, 836, 776, 738, 699 cm^{–1}.

¹H NMR (CDCl₃, 500 MHz): δ = 7.36–7.26 (m, 5 H, CH-Ph), 5.21 (dd, *J* = 9.8, 9.9 Hz, 1 H, 3''-H), 5.10–5.03 (m, 2 H, 2''-H, 4''-H), 4.99 (dd, *J* = 8.0, 9.7 Hz, 1 H, 2''-H), 4.92 (dd, *J* = 3.1, 10.2 Hz, 1 H, 3'-H), 4.57–4.50 (m, 3 H, CH₂-Bn, 1''-H), 4.40 (d, *J* = 7.9 Hz, 1 H, 1'-H), 4.16 (dd, *J* = 4.4, 12.4 Hz, 1 H, 6''-H^a), 4.12 (d, *J* = 2.9 Hz, 1 H, 4'-H), 4.03 (dd, *J* = 2.1, 12.2 Hz, 1 H, 6''-H^b), 3.84 (dt, *J* = 6.4, 9.8 Hz, 1 H, 1-H^a), 3.74 (dd, *J* = 4.7, 9.3 Hz, 1 H, 6'-H^a), 3.71–3.63 (m, 2 H, 11-H^a, 5'-H), 3.63–3.58 (m, 3 H, 11-H^b, 6'-H^b, 5''-H), 3.44 (dt, *J* = 7.0, 9.7 Hz, 1 H, 1-H^b), 2.16 (s, 3 H, OAc), 2.12 (s, 3 H, OAc), 2.02 (s, 6 H, OAc), 2.01 (s, 6 H, OAc), 1.55–1.44 (m, 4 H, 2-H, 10-H), 1.35–1.23 (m, 14 H, 3-H to 9-H), 0.90 (s, 9 H, *t*Bu), 0.04 (s, 6 H, Me).

¹³C NMR (CDCl₃, 125 MHz): δ = 170.7, 170.6, 170.5, 169.7, 169.6, 169.5, 128.6, 127.8, 127.6, 101.4, 100.9, 74.5, 73.61, 73.58, 73.5, 72.8, 71.9, 71.5, 69.7, 69.6, 69.3, 68.4, 63.5, 61.8, 33.0, 29.80, 29.77, 29.73, 29.60, 29.56, 29.5, 26.1, 26.01, 25.95, 21.0, 20.9, 20.8, 20.74, 20.68, –5.1.

HRMS (ESI): *m/z* [M + Na]⁺ calcd for C₄₈H₇₆O₁₈SiNa: 991.46931; found: 991.46599.

(11-(tert-Butyldimethylsilyloxy)undecyl)-2'',3'',4'',6''-tetra-O-acetyl-β-D-glucopyranosyl-(1→4)-2',3'-di-O-acetyl-1'-O-β-D-galactopyranoside (19)

To a solution of **18** (0.478 g, 0.49 mmol, 1.00 equiv) in EtOAc (13 mL) was added 5% palladium on charcoal (0.096 g) at r.t. The suspension was saturated with H₂ and stirred under an atmosphere of H₂ for 2.5 h. After consumption of the starting material the mixture was filtered through Celite and the filtrate was concentrated under reduced pressure to afford title compound **19**, which was used without further purification.

Yield: 0.432 g (0.493 mmol, quant.); colourless waxy foam; $[\alpha]_D^{25}$ +2.1 (*c* = 1.00 CHCl₃); *R*_f = 0.40 (cyclohexane/EtOAc, 1:1).

IR (ATR): 3549, 2929, 2856, 1751, 1470, 1432, 1369, 1224, 1170, 1040, 907, 836, 775 cm^{–1}.

¹H NMR (CDCl₃, 500 MHz): δ = 5.23 (dd, *J* = 9.5, 9.6 Hz, 1 H, 3''-H), 5.08–5.01 (m, 2 H, 2''-H, 4''-H), 4.97 (dd, *J* = 9.7, 9.8 Hz, 1 H, 4''-H), 4.93 (dd, *J* = 3.2, 10.4 Hz, 1 H, 3'-H), 4.53 (d, *J* = 8.1 Hz, 1 H, 1''-H), 4.41 (d, *J* = 7.9 Hz, 1 H, 1'-H), 4.36 (dd, *J* = 2.2, 12.3 Hz, 1 H, 6''-H^a), 4.20 (d, *J* = 2.9 Hz, 1 H, 4'-H), 3.95 (dd, *J* = 7.3, 12.2 Hz, 1 H, 6''-H^b), 3.85–3.78 (m, 2 H, 6'-H^a, 1-H^a), 3.72 (ddd, *J* = 2.3, 7.3, 9.8 Hz, 1 H, 5''-H), 3.67–3.61 (m, 1 H, 6'-H^b), 3.61–3.55 (m, 3 H, 11-H, 5'-H), 3.42 (dt, *J* = 6.7, 9.7 Hz, 1 H, 1-H^b), 2.98–2.86 (m, 1 H, OH), 2.16 (s, 3 H, OAc), 2.12 (s, 3 H, OAc), 2.10 (s, 3 H, OAc), 2.04 (s, 3 H, OAc), 2.02 (s, 6 H, OAc), 1.57–1.46 (m, 4 H, 2-H, 10-H), 1.31–1.24 (m, 14 H, 3-H to 9-H), 0.90 (s, 9 H, *t*Bu), 0.05 (s, 6 H, Me).

¹³C NMR (CDCl₃, 125 MHz): δ = 170.7, 170.50, 170.47, 169.6, 169.53, 169.45, 102.1, 101.0, 74.2, 73.43, 73.41, 72.5, 72.1, 71.0, 69.8, 69.6, 68.7, 63.5, 62.4, 59.3, 33.0, 29.8, 29.75, 29.72, 29.6, 29.54, 29.51, 26.1, 25.98, 25.95, 21.0, 20.9, 20.8, 20.75, 20.73, 20.6, 18.5, –5.1.

HRMS (ESI): *m/z* [M + Na]⁺ calcd for C₄₁H₇₀O₁₈SiNa: 901.42236; found: 901.42146.

(11-(tert-Butyldimethylsilyloxy)undecyl)-2'',3'',4'',6''-tetra-O-acetyl-β-D-glucopyranosyl-(1→4)-2',3'-di-O-acetyl-1'-O-β-D-galactopyranosiduronic Acid (20)

A solution of **19** (0.374 g, 0.425 mmol, 1.00 equiv) in CH₂Cl₂ (5.7 mL) and H₂O (2.8 mL) was treated with BAIB (0.301 g, 0.936 mmol, 2.20 equiv) and TEMPO (0.013 g, 0.085 mmol, 0.20 equiv) at r.t. The mixture was stirred for 1 h, diluted with EtOAc, washed with aqueous 10 wt% Na₂S₂O₃ solution and the aqueous phase was extracted with EtOAc (3 × 15 mL). The combined organic phases were washed with brine, dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. The residue was purified by column chromatography (cyclohexane/EtOAc, 7:3 → CH₂Cl₂/MeOH, 97:3) to afford title compound **20**.

Yield: 0.331 g (0.371 mmol, 86%); colourless foam; mp 73–74 °C $[\alpha]_D^{25}$ –19.0 (*c* = 1.00 CHCl₃); *R*_f = 0.38 (CH₂Cl₂/MeOH, 9:1).

IR (ATR): 2928, 2856, 1750, 1631, 1595, 1566, 1431, 1367, 1220, 1169, 1041, 930, 835, 776, 687, 661, 601 cm^{–1}.

¹H NMR (CDCl₃, 500 MHz): δ = 5.19 (dd, *J* = 9.5, 9.6 Hz, 1 H, 3''-H), 5.16–5.07 (m, 2 H, 2''-H, 4''-H), 4.96 (dd, *J* = 8.1, 9.7 Hz, 1 H, 2''-H), 4.92 (dd, *J* = 3.1, 12.4 Hz, 1 H, 4'-H), 4.83 (dd, *J* = 2.3, 12.4 Hz, 1 H, 6''-H^a), 4.50 (d, *J* = 8.0 Hz, 1 H, 1'-H), 4.45 (d, *J* = 7.9 Hz, 1 H, 1''-H), 4.42 (d, *J* = 2.7 Hz, 1 H, 5'-H), 4.14 (s, 1 H, 3''-H), 3.92 (dt, *J* = 6.3, 9.5 Hz, 1 H, 1-H^a), 3.80 (dd, *J* = 2.6, 12.4 Hz, 1 H, 6''-H^b), 3.62–3.56 (m, 3 H, 11-H, 5''-H), 3.44 (dt, *J* = 7.0, 9.4 Hz, 1 H, 1-H^b), 2.19 (s, 3 H, OAc), 2.13 (s, 3 H, OAc), 2.11 (s, 3 H, OAc), 2.03 (s, 3 H, OAc), 2.02 (s, 3 H, OAc), 2.01 (s, 3 H, OAc), 1.60–1.46 (m, 4 H, 2-H, 10-H), 1.35–1.19 (m, 14 H, 3-H to 9-H), 0.89 (s, 9 H, *t*Bu), 0.04 (s, 6 H, Me).

¹³C NMR (CDCl₃, 125 MHz): δ = 173.7, 170.7, 170.4, 169.4, 169.3, 169.2, 166.5, 101.4, 100.7, 76.1, 73.02, 72.99, 72.7, 71.7, 71.3, 70.2, 68.8, 68.1, 63.5, 60.2, 33.0, 29.8, 29.74, 29.70, 29.6, 29.5, 29.4, 26.1, 25.9, 21.4, 20.9, 20.83, 20.81, 20.78, 20.6, 18.5, –5.1.

HRMS (ESI): *m/z* [M + Na]⁺ calcd for C₄₁H₆₈O₁₉SiNa: 915.40163; found: 915.39962.

Methyl ((11-(tert-Butyldimethylsilyloxy)undecyl)-2'',3'',4'',6''-tetra-O-acetyl-β-D-glucopyranosyl-(1→4)-2',3'-di-O-acetyl-1'-O-β-D-galactopyranoside)uronate (21)

A solution of **20** (0.309 g, 0.346 mmol, 1.00 equiv) in DMF (7 mL) was treated with dried K₂CO₃ (0.057 g, 0.415 mmol, 1.20 equiv) and MeI (28.0 μL, 0.450 mmol, 1.30 equiv) at 0 °C. The mixture was warmed to r.t., stirred for 2.5 h, diluted with EtOAc, washed with aqueous 10 wt% Na₂S₂O₃ solution, and extracted with EtOAc (3 × 15 mL). The combined organic phases were washed with H₂O and brine, dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. The residue was purified by column chromatography (cyclohexane/EtOAc, 1:1) to afford title compound **21**.

Yield: 0.300 g (0.331 mmol, 96%); colourless oil; $[\alpha]_D^{25}$ –5.0 (*c* = 1.00 CHCl₃); *R*_f = 0.83 (cyclohexane/EtOAc, 1:4).

IR (ATR): 2932, 2856, 1751, 1440, 1370, 1222, 1173, 1091, 1044, 837, 774 cm^{–1}.

¹H NMR (CDCl₃, 500 MHz): δ = 5.22 (dd, *J* = 9.6, 9.7 Hz, 1 H, 3''-H), 5.16 (dd, *J* = 8.0, 10.3 Hz, 1 H, 2''-H), 5.01 (dd, *J* = 9.7, 9.9 Hz, 1 H, 4''-H), 4.96 (dd, *J* = 3.1, 10.3 Hz, 1 H, 3'-H), 4.93 (dd, *J* = 8.1, 9.6 Hz, 1 H, 2''-H), 4.54 (d, *J* = 8.1 Hz, 1 H, 1''-H), 4.45–4.42 (m, 1 H, 4'-H), 4.42 (d, *J* = 8.0 Hz, 1 H, 1'-H), 4.31 (dd, *J* = 4.9, 12.4 Hz, 1 H, 6''-H^a), 4.17 (d, *J* = 0.8 Hz, 1 H, 5'-H), 4.00 (dd, *J* = 2.1, 12.4 Hz, 1 H, 6''-H^b), 3.94 (dt, *J* = 6.1, 9.5 Hz,

¹H, 1-H^a), 3.75 (s, 3 H, OMe), 3.63 (ddd, *J* = 2.1, 4.9, 9.9 Hz, 1 H, 5"-H), 3.60 (t, *J* = 6.6 Hz, 2 H, 11-H), 3.45 (dt, *J* = 6.7, 9.5 Hz, 1 H, 1-H^b), 2.14 (s, 6 H, OAc), 2.09 (s, 3 H, OAc), 2.03 (s, 3 H, OAc), 2.01 (s, 6 H, OAc), 1.55–1.47 (m, 4 H, 2-H, 10-H), 1.34–1.23 (m, 14 H, 3-H to 9-H), 0.90 (s, 9 H, tBu), 0.05 (s, 6 H, Me).

¹³C NMR (CDCl₃, 125 MHz): δ = 170.8, 170.6, 170.4, 169.5, 169.4, 169.3, 166.9, 101.5, 100.8, 75.7, 73.9, 73.0, 72.8, 71.9, 71.6, 70.1, 68.9, 68.2, 63.5, 61.9, 52.6, 33.0, 29.8, 29.75, 29.72, 29.6, 29.5, 29.4, 26.1, 26.0, 20.9, 20.84, 20.81, 20.72, 20.66, –5.1.

HRMS (ESI): *m/z* [M + Na]⁺ calcd for C₄₂H₇₀O₁₉SiNa: 929.41728; found: 929.41515.

Methyl ((11-Hydroxyundecyl)-2",3",4"6"-tetra-*O*-acetyl- β -D-glucopyranosyl-(1 \rightarrow 4)-2',3'-di-*O*-acetyl-1'-*O*- β -D-galactopyranoside)uronate (22)

A solution of **21** (0.300 g, 0.331 mmol, 1.00 equiv) in THF (1.7 mL), H₂O (1.7 mL) and AcOH (5.0 mL) was stirred for 2 h at r.t. After consumption of the starting silyl ether, the mixture was diluted with EtOAc, washed with saturated aqueous NaHCO₃ solution, and extracted with EtOAc (3 \times 15 mL). The combined organic phases were washed with brine, dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. The remaining residue was purified by column chromatography (cyclohexane/EtOAc, 1:1) to afford alcohol **22**.

Yield: 0.262 g (0.331 mmol, quant.); colourless oil; [α]_D²⁵ –5.9 (*c* = 1.00 CHCl₃); *R*_f = 0.45 (cyclohexane/EtOAc, 1:4).

IR (ATR): 2930, 2857, 2154, 1749, 1437, 1369, 1223, 1168, 1043, 923, 605, 583, 568 cm^{–1}.

¹H NMR (CDCl₃, 500 MHz): δ = 5.22 (dd, *J* = 9.5, 9.7 Hz, 1 H, 3"-H), 5.16 (dd, *J* = 8.0, 10.4 Hz, 1 H, 2'-H), 5.01 (dd, *J* = 9.5, 9.9 Hz, 1 H, 4"-H), 4.95 (dd, *J* = 3.1, 10.4 Hz, 1 H, 3'-H), 4.93 (dd, *J* = 8.1, 9.7 Hz, 1 H, 2"-H), 4.54 (d, *J* = 8.1 Hz, 1 H, 1"-H), 4.45–4.42 (m, 1 H, 4'-H), 4.42 (d, *J* = 8.0 Hz, 1 H, 1'-H), 4.31 (dd, *J* = 4.7, 12.4 Hz, 1 H, 6"-H^a), 4.16 (d, *J* = 0.9 Hz, 1 H, 5'-H), 4.00 (dd, *J* = 2.1, 12.4 Hz, 1 H, 6"-H^b), 3.94 (dt, *J* = 6.4, 9.5 Hz, 1 H, 1-H^a), 3.75 (s, 3 H, OMe), 3.65 (t, *J* = 6.6 Hz, 2 H, 11-H), 3.63 (ddd, *J* = 2.1, 4.7, 9.9 Hz, 1 H, 5"-H), 3.46 (dt, *J* = 6.8, 9.5 Hz, 1 H, 1-H^b), 2.13 (s, 6 H, OAc), 2.08 (s, 3 H, OAc), 2.03 (s, 3 H, OAc), 2.01 (s, 6 H, OAc), 1.59–1.54 (m, 4 H, 2-H, 10-H), 1.34–1.25 (m, 14 H, 3-H to 9-H).

¹³C NMR (CDCl₃, 125 MHz): δ = 203.1, 170.8, 170.6, 170.4, 169.50, 169.45, 169.3, 166.9, 101.5, 100.8, 75.7, 73.9, 73.0, 72.8, 71.9, 71.6, 70.1, 69.9, 68.2, 63.2, 61.9, 52.6, 32.9, 29.7, 29.7, 29.5, 29.44, 29.41, 25.91, 25.85, 20.9, 20.85, 20.83, 20.81, 20.72, 20.66.

HRMS (ESI): *m/z* [M + Na]⁺ calcd for C₃₆H₅₆O₁₉Na: 815.33080; found: 815.32949.

Methyl ((11-Oxoundecyl)-2",3",4"6"-tetra-*O*-acetyl- β -D-glucopyranosyl-(1 \rightarrow 4)-2',3'-di-*O*-acetyl-1'-*O*- β -D-galactopyranoside)uronate (6)

A mixture of **22** (0.262 g, 0.331 mmol, 1.00 equiv), CH₂Cl₂ (7.2 mL), and DMP (0.210 g, 0.496 mmol, 1.50 equiv) was stirred at r.t. for 1 h. After consumption of the starting alcohol, the mixture was diluted with CH₂Cl₂ and washed with an aqueous 1:1 mixture of saturated NaHCO₃ and 10 wt% Na₂S₂O₃. The aqueous phase was extracted with CH₂Cl₂ (3 \times 15 mL), the combined organic phases were washed with brine, dried over anhydrous Na₂SO₄ and concentrated under reduced pressure to afford aldehyde **6**, which was used without further purification.

Yield: 0.260 g (0.331 mmol, quant.); colourless foam; mp 66–67 °C; [α]_D²⁵ –4.4 (*c* = 1.00 CHCl₃); *R*_f = 0.48 (cyclohexane/EtOAc, 3:7).

IR (ATR): 2929, 2856, 1746, 1439, 1368, 1220, 1169, 1122, 1084, 1041, 925, 778 cm^{–1}.

¹H NMR (CDCl₃, 500 MHz): δ = 9.77 (t, *J* = 1.9 Hz, 1 H, 11-H), 5.22 (dd, *J* = 9.5, 9.6 Hz, 1 H, 3"-H), 5.16 (dd, *J* = 8.0, 10.2 Hz, 1 H, 2'-H), 5.01 (dd, *J* = 9.6, 9.9 Hz, 1 H, 4"-H), 4.96 (dd, *J* = 7.2, 10.2 Hz, 1 H, 3'-H), 4.93 (dd, *J* = 8.1, 9.5 Hz, 1 H, 2"-H), 4.54 (d, *J* = 8.1 Hz, 1 H, 1"-H), 4.46–4.43 (m, 1 H, 4'-H), 4.43 (d, *J* = 8.1 Hz, 1 H, 1'-H), 4.31 (dd, *J* = 4.9, 12.4 Hz, 1 H, 6"-H^a), 4.16 (d, *J* = 0.7 Hz, 1 H, 5'-H), 4.00 (dd, *J* = 2.0, 12.4 Hz, 1 H, 6"-H^b), 3.94 (dt, *J* = 6.3, 9.4 Hz, 1 H, 1-H^a), 3.75 (s, 3 H, OMe), 3.63 (ddd, *J* = 2.1, 4.9, 9.9 Hz, 1 H, 5"-H), 3.45 (dt, *J* = 7.0, 9.5 Hz, 1 H, 1-H^b), 2.43 (dt, *J* = 2.0, 7.4 Hz, 2 H, 10-H), 2.14 (s, 6 H, OAc), 2.09 (s, 3 H, OAc), 2.03 (s, 3 H, OAc), 2.01 (s, 6 H, OAc), 1.64–1.52 (m, 4 H, 2-H, 9-H), 1.35–1.24 (m, 12 H, 3-H to 8-H).

¹³C NMR (CDCl₃, 125 MHz): δ = 202.6, 170.8, 170.6, 170.4, 169.5, 169.4, 169.3, 166.9, 101.5, 100.8, 75.7, 73.9, 73.0, 71.9, 71.6, 70.1, 68.9, 68.2, 61.9, 52.6, 29.6, 29.47, 29.43, 29.41, 29.3, 25.9, 22.2, 20.9, 20.8, 20.72, 20.66.

HRMS (ESI): *m/z* [M + Na]⁺ calcd for C₃₆H₅₄O₁₉Na: 813.31515; found: 813.31377.

Methyl ((*Z*)-23-Carboxy-tricos-11-enyl-(2",3",4"6"-tetra-*O*-acetyl- β -D-glycopyranosyl)-(1 \rightarrow 4)-2',3'-di-*O*-acetyl-1'-*O*- β -D-galactopyranoside)uronate (23)

NaHMDS (1 M in THF, 0.36 mL, 0.36 mmol, 3.00 equiv) was added dropwise to a solution of **7** (0.088 g, 0.16 mmol, 1.30 equiv) in THF (2 mL) at –78 °C. The orange solution was warmed to r.t., stirred for 30 min, and re-cooled to –78 °C. A solution of **6** (0.096 g, 0.12 mmol, 1.00 equiv) in THF (2.5 mL) was added dropwise at this temperature, the resulting mixture was slowly warmed to r.t. over 2 h, and the reaction was quenched by adding 0.1 M aqueous HCl when the starting aldehyde was consumed. The mixture was extracted with EtOAc (3 \times 5 mL), and the combined organic phases were washed with brine, dried over Na₂SO₄ and the solvent was evaporated under reduced pressure. The residue was purified by column chromatography (cyclohexane/EtOAc, 2:3) to afford alkene **23**.

Yield: 0.070 g (0.071 mmol, 58%); colourless oil; *R*_f = 0.26 (cyclohexane/EtOAc, 1:3).

IR (ATR): 2926, 2855, 1746, 1438, 1367, 1216, 1164, 1120, 1086, 1036, 927, 779, 749, 722, 695, 599, 580 cm^{–1}.

¹H NMR (CDCl₃, 500 MHz): δ = 5.38–5.29 (m, 2 H, 11-H, 12-H), 5.21 (dd, *J* = 9.3, 9.7 Hz, 1 H, 3"-H), 5.14 (dd, *J* = 8.0, 10.3 Hz, 1 H, 2'-H), 5.00 (dd, *J* = 9.7, 9.8 Hz, 1 H, 4"-H), 4.98–4.91 (m, 2 H, 3'-H, 2"-H), 4.53 (d, *J* = 8.1 Hz, 1 H, 1"-H), 4.45–4.41 (m, 2 H, 1'-H, 4'-H), 4.30 (dd, *J* = 4.8, 12.4 Hz, 1 H, 6"-H^a), 4.17 (d, *J* = 0.8 Hz, 1 H, 5'-H), 3.99 (dd, *J* = 1.9, 12.4 Hz, 1 H, 6"-H^b), 3.93 (dt, *J* = 6.2, 9.3 Hz, 1 H, 1-H^a), 3.74 (s, 3 H, OMe), 3.62 (ddd, *J* = 2.1, 4.8, 10.2 Hz, 1 H, 5"-H), 3.44 (dt, *J* = 6.9, 9.5 Hz, 1 H, 1-H^b), 2.34 (t, *J* = 7.5 Hz, 2 H, 23-H), 2.13 (s, 6 H, OAc), 2.08 (s, 3 H, OAc), 2.07–1.97 (m, 13 H, OAc, 10-H, 13-H), 1.67–1.53 (m, 4 H, 2-H, 22-H), 1.39–1.20 (m, 30 H, 3-H to 9-H and 14-H to 21-H).

¹³C NMR (CDCl₃, 125 MHz): δ = 170.7, 170.5, 170.4, 169.4, 169.2, 166.9, 129.9, 101.4, 100.6, 75.5, 73.7, 72.9, 72.7, 71.8, 71.5, 70.0, 68.8, 68.1, 65.6, 61.8, 52.5, 34.5, 33.4, 29.8, 29.7, 29.64, 29.61, 29.58, 29.57, 29.52, 29.49, 29.45, 29.40, 29.33, 29.30, 29.27, 29.09, 29.06, 27.21, 27.19, 25.8, 24.8, 20.8, 20.72, 20.70, 20.68, 20.6, 20.5.

HRMS (ESI): *m/z* [M + Na]⁺ calcd for C₄₉H₇₈O₂₀Na: 1009.49787; found: 1009.49768.

Methyl ((Z)-Tricos-11-enyl-(2,2-dimethyl-1,3-dioxane-4,6-dione)-(2'',3'',4'',6''-tetra-O-acetyl-β-D-glycopyranosyl)-(1→4)-2',3'-di-O-acetyl-1'-O-β-D-galactopyranoside)uronate (4)

To a solution of **23** (0.065 g, 0.066 mmol, 1.00 equiv) in CH_2Cl_2 (2.5 mL) were added DMAP (0.0084 g, 0.069 mmol, 1.05 equiv), EDCI (0.014 g, 0.073 mmol, 1.10 equiv), and Meldrum's acid (0.011 g, 0.073 mmol, 1.10 equiv) at 0 °C. The mixture was warmed to r.t. and stirred for 16 h. The mixture was diluted with EtOAc, the reaction was quenched by addition of 0.1 M aqueous HCl and the mixture was extracted with EtOAc (3 × 5 mL). The combined organic phases were washed with brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by a quick column chromatography over a short plug of silica (cyclohexane/EtOAc, 2:3) to afford title compound **4**, which was used without further purification.

Yield: 0.047 g (0.045 mmol, 68%); colourless oil; R_f = 0.16 (cyclohexane/EtOAc, 2:3).

Fully Protected Ancorinoside D (24)

A solution of **4** (0.034 g, 0.031 mmol, 1.00 equiv) and **5** (0.0064 g, 0.037 mmol, 1.20 equiv) in toluene (3 mL) was heated at reflux for 4 h. The mixture was cooled to r.t., the solvent was evaporated under reduced pressure, and the remaining residue was purified by column chromatography (cyclohexane/EtOAc, 1:3 → $\text{CH}_2\text{Cl}_2/\text{MeOH}$, 9:1) to afford title compound **24**.

Yield: 0.024 g (0.0208 mmol, 68%); pale-reddish oil; $[\alpha]_{\text{D}}^{25}$ +15.5 (c = 1.00 CHCl_3); R_f = 0.04 (cyclohexane/EtOAc, 1:3).

IR (ATR): 2925, 2853, 1748, 1612, 1490, 1437, 1368, 1223, 1169, 1125, 1087, 1043 cm^{-1} .

^1H NMR (CDCl_3 , 500 MHz): δ = 5.38–5.29 (m, 2 H, 20-H, 21-H), 5.20 (dd, J = 9.5, 9.6 Hz, 1 H, 3''-H), 5.13 (dd, J = 8.1, 10.2 Hz, 1 H, 2'-H), 5.02–4.90 (m, 3 H, 3'-H, 2''-H, 4''-H), 4.52 (d, J = 8.3 Hz, 1 H, 1''-H), 4.46–4.40 (m, 2 H, 1'-H, 4'-H), 4.30 (dd, J = 4.9, 12.5 Hz, 1 H, 6''-H^a), 4.16 (s, 1 H, 5'-H), 3.99 (dd, J = 1.7, 12.5 Hz, 1 H, 6''-H^b), 3.92 (dt, J = 6.2, 9.3 Hz, 1 H, 31-H^a), 3.85–3.78 (m, 1 H, 5-H), 3.74 (s, 3 H, OMe), 3.73–3.68 (m, 3 H, OMe), 3.62 (ddd, J = 2.1, 4.7, 10.0 Hz, 1 H, 5''-H), 3.44 (dt, J = 7.0, 9.4 Hz, 1 H, 31-H^b), 2.98–2.71 (m, 6 H, NMe, 6-H^a, 9-H), 2.62–2.49 (m, 1 H, 6-H^b), 2.09 (s, 6 H, OAc), 2.04 (s, 3 H, OAc), 2.03–1.97 (m, 13 H, OAc, 19-H, 22-H), 1.62–1.42 (m, 4 H, 10-H, 30-H), 1.40–1.14 (m, 30 H, 11-H to 18-H and 23-H to 29-H).

^{13}C NMR (CDCl_3 , 125 MHz): δ = 170.8, 170.6, 170.4, 169.54, 169.49, 169.3, 166.9, 130.0, 101.5, 100.7, 97.7, 75.6, 73.8, 73.0, 72.8, 71.9, 71.6, 70.1, 68.9, 68.2, 61.9, 52.6, 52.1, 29.96, 29.93, 29.89, 29.87, 29.81, 29.78, 29.75, 29.73, 29.54, 29.53, 29.48, 29.4, 27.4, 27.38, 27.36, 25.9, 20.9, 20.83, 20.81, 20.79, 20.70, 20.64.

HRMS (ESI): m/z [$\text{M} + \text{Na}$]⁺ calcd for $\text{C}_{57}\text{H}_{87}\text{NO}_{23}\text{Na}$: 1176.55611; found: 1176.55359.

Ancorinoside D Methyl Ester (25)

A solution of **24** (13.36 mg, 0.01157 mmol, 1.00 equiv) in CH_2Cl_2 (1.8 mL) and MeOH (0.2 mL) was treated with methanolic NaOH (2 M, 0.069 mL, 0.1389 mmol, 12.00 equiv) and stirred at r.t. for 30 min. The mixture was acidified by addition of DOWEX 50WX8, which was filtered off again, and the solvent was evaporated under reduced pressure to afford the title ester **25**, which was sufficiently pure for its analytical characterisation.

Yield: 9.97 mg (0.01123 mmol, 97%); pale-reddish oil; $[\alpha]_{\text{D}}^{25}$ +41.8 (c = 0.50 MeOH).

IR (ATR): 3400, 2923, 2854, 1739, 1712, 1626, 1485, 1454, 1437, 1401, 1372, 1299, 1258, 1227, 1171, 1122, 1110, 1080, 1035, 1010 cm^{-1} .

^1H NMR (CD_3OD , 500 MHz): δ = 5.34 (t, J = 5.0 Hz, 2 H, 20-H, 21-H), 4.48 (d, J = 7.8 Hz, 1 H, 1''-H), 4.34 (d, J = 3.0 Hz, 1 H, 4'-H), 4.28 (d, J = 7.7 Hz, 1 H, 1'-H), 4.24 (s, 1 H, 5'-H), 4.08 (dt, J = 4.9, 8.3 Hz, 1 H, 5-H), 3.94 (dt, J = 6.8, 9.4 Hz, 1 H, 31-H^a), 3.85 (dd, J = 2.2, 11.9 Hz, 1 H, 6''-H^a), 3.67–3.62 (m, 5 H, 3'-H, 6''-H^b, OMe), 3.58–3.52 (m, 2 H, 2'-H, 31-H^b), 3.36–3.26 (m, 2 H, 3''-H, 4''-H), 3.24–3.18 (m, 2 H, 2''-H, 5''-H), 2.95 (d, J = 12.2 Hz, 3 H, NMe), 2.93–2.88 (m, 2 H, 9-H), 2.88–2.76 (m, 2 H, 6-H), 2.03 (q, J = 6.0 Hz, 4 H, 19-H, 22-H), 1.70–1.58 (m, 4 H, 10-H, 30-H), 1.43–1.27 (m, 30 H, 11-H to 18-H and 23-H to 29-H).

^{13}C NMR (CD_3OD , 125 MHz): δ = 196.4, 188.0, 173.1, 171.9, 171.8, 171.7, 130.9, 106.0, 104.7, 102.4, 80.9, 78.1, 77.9, 75.7, 75.6, 74.9, 74.6, 72.5, 71.3, 71.2, 64.2, 62.6, 52.4, 34.4, 34.3, 33.2, 30.87, 30.79, 30.71, 30.68, 30.59, 30.54, 30.36, 30.28, 30.22, 28.13, 28.09, 27.23, 27.11, 26.9.

HRMS (ESI): m/z [$\text{M} + \text{Na}$]⁺ calcd for $\text{C}_{48}\text{H}_{73}\text{NO}_{17}\text{Na}$: 910.47707; found: 910.47778.

Supporting Information

Supporting information for this article is available online at <https://doi.org/10.1055/s-0037-1610287>.

References

- (1) Fujita, M.; Nakao, Y.; Matsunaga, S.; Seiki, M.; Itoh, Y.; van Soest, R. W. M.; Fusetani, N. *Tetrahedron* **2001**, 57, 1229.
- (2) (a) Gialeli, C.; Theocharis, A. D.; Karamanos, N. K. *FEBS J.* **2011**, 278, 16. (b) Ziyad, S.; Iruela-Arispe, M. L. *Genes Cancer* **2011**, 2, 1085. (c) Sounni, N. E.; Devy, L.; Hajitou, A.; Frankenne, D.; Munaut, C.; Gilles, C.; Deroanne, C.; Thompson, E. W.; Foidart, J. M.; Noel, A. *FASEB J.* **2002**, 16, 555.
- (3) Ohta, S.; Ohta, E.; Ikegami, S. *J. Org. Chem.* **1997**, 62, 6452.
- (4) Petermichl, M.; Schobert, R. *Chem. Eur. J.* **2017**, 23, 14743.
- (5) (a) Ley, S. V.; Smith, S. C.; Woodward, P. R. *Tetrahedron* **1992**, 48, 1145. (b) Pronin, S. V.; Kozmin, S. A. *J. Am. Chem. Soc.* **2010**, 132, 14394. (c) Loscher, S.; Schobert, R. *Chem. Eur. J.* **2013**, 19, 10619.
- (6) (a) Girlanda-Junges, C.; Keyling-Bilger, F.; Schmitt, G.; Luu, B. *Tetrahedron* **1998**, 54, 7735. (b) Mori, K.; Nishio, H. *Liebigs Ann. Chem.* **1991**, 253.
- (7) (a) Adrio, L. A.; Hii, K. K. *Eur. J. Org. Chem.* **2011**, 1852. (b) Epp, J. B.; Widlanski, T. S. *J. Org. Chem.* **1999**, 64, 293.
- (8) (a) Müller, S.; Schmidt, R. R. *Helv. Chim. Acta* **1993**, 76, 616. (b) Thurnhofer, S.; Vetter, W. *Tetrahedron* **2007**, 63, 1140.
- (9) Huang, Z.; Zhang, Y.-M.; Cheng, Q.; Zhang, J.; Liu, Y.-H.; Wang, B.; Yu, X.-Q. *J. Mater. Chem. B* **2016**, 4, 5575.
- (10) McDougal, P. G.; Rico, J. G.; Oh, Y.-I.; Condon, B. D. *J. Org. Chem.* **1986**, 51, 3388.
- (11) (a) Mitchell, A. J.; Dunham, N. P.; Martinie, R. J.; Berman, J. A.; Pollock, C. J.; Hu, K.; Allen, B. D.; Chang, W.-C.; Silakov, A.; Bollinger, J. M. Jr.; Krebs, C.; Boal, A. K. *J. Am. Chem. Soc.* **2017**, 139, 13830. (b) Reid, C. M.; Fanning, K. N.; Fowler, L. S.; Sutherland, A. *Tetrahedron* **2015**, 71, 245.
- (12) Wunder, A.; Rothmund, M.; Schobert, R. *Tetrahedron* **2018**, 74, 5138.
- (13) Frérot, E.; Coste, J.; Poncet, J.; Jouin, P.; Castro, B. *Tetrahedron Lett.* **1992**, 33, 2815.
- (14) (a) Schmidt, R. R.; Michel, J. *Angew. Chem., Int. Ed. Engl.* **1980**, 19, 731. (b) Schmidt, R. R. *Angew. Chem., Int. Ed. Engl.* **1986**, 25, 212.

- (15) Lewis, A. R.; Reber, K. P. *Tetrahedron Lett.* **2016**, 57, 1083.
- (16) (a) Ren, J.-L.; Zhang, X.-Y.; Yu, B.; Wang, X.-X.; Shao, K.-P.; Zhu, X.-G.; Liu, H.-M. *Eur. J. Med. Chem.* **2015**, 93, 321. (b) Bruckner, S.; Haase, R.; Schobert, R. *Chemistry* **2017**, 23, 5692.
- (17) (a) Jouin, P.; Castro, B. *J. Chem. Soc., Perkin Trans. 1* **1987**, 1177. (b) Xu, F.; Armstrong, J. D. III.; Zhou, G. X.; Simmons, B.; Hughes, D.; Ge, Z.; Grabowski, E. J. J. *Am. Chem. Soc.* **2004**, 126, 13002.
- (18) (a) Pak, C. S.; Yang, H. C.; Choi, E. B. *Synthesis* **1992**, 1213. (b) Ivanov, A. S. *Chem. Soc. Rev.* **2008**, 37, 789.
- (19) Lovmo, K.; Dütz, S.; Harras, M.; Haase, R. G.; Milius, W.; Schobert, R. *Tetrahedron Lett.* **2017**, 58, 4796.
- (20) (a) Hartmann, O.; Kalesse, M. *Angew. Chem. Int. Ed.* **2014**, 53, 7335. (b) Corey, E. J.; Székely, I.; Shiner, C. S. *Tetrahedron Lett.* **1977**, 3529.
- (21) (a) Theodorou, V.; Skobridis, K.; Tzakos, G. A.; Ragoussis, V. *Tetrahedron Lett.* **2007**, 48, 8230. (b) Theodorou-Kassioumis, V.; Biris, N.; Sakarellos, C.; Tsikaris, V. *Tetrahedron Lett.* **2001**, 42, 7703.
- (22) (a) Lovrić, M.; Cepanec, I.; Litvić, M.; Bartolinčić, A.; Vinković, V. *Croat. Chem. Acta* **2007**, 80, 109. (b) Laganis, E. D.; Chenard, B. L. *Tetrahedron Lett.* **1984**, 25, 5831. (c) Dean, P. D. *J. Chem. Soc.* **1965**, 6655.
- (23) Fulmer, G. R.; Miller, A. J. M.; Sherden, N. H.; Gottlieb, H. E.; Nudelman, A.; Stoltz, B. M.; Bercaw, J. E.; Goldberg, K. I. *Organometallics* **2010**, 29, 2176.
- (24) Kim, J.; Novak, B. M. *Macromolecules* **2004**, 37, 8286.

Supporting Information

A Synthetic Route to the MT1-MMP Inhibitor Ancorinoside D

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| NMR Spectra | 2-20 |

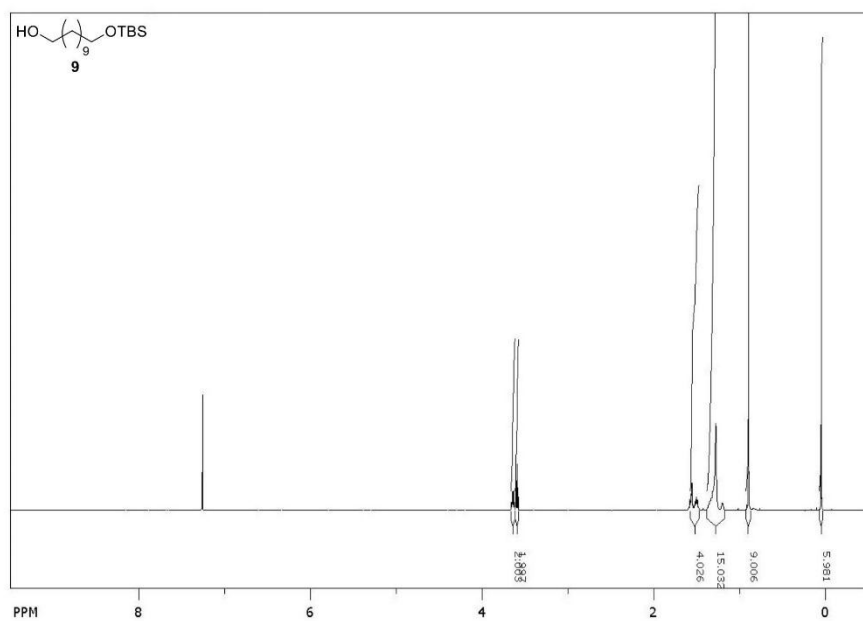


Figure 1 11-(tert-Butyldimethylsilyloxy)undecan-1-ol (9), ¹H NMR (500 MHz, CDCl₃).

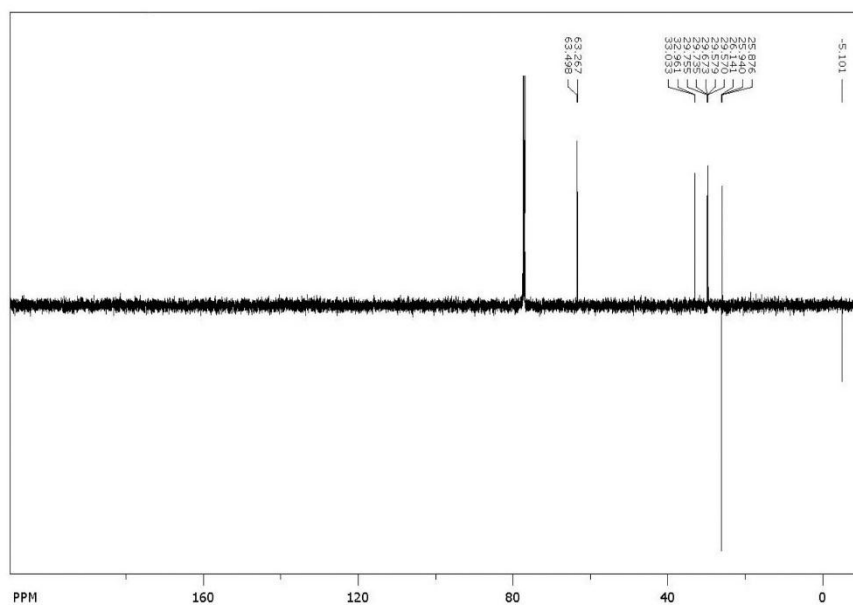


Figure 2 11-(tert-Butyldimethylsilyloxy)undecan-1-ol (9), ¹³C NMR (125 MHz, CDCl₃).

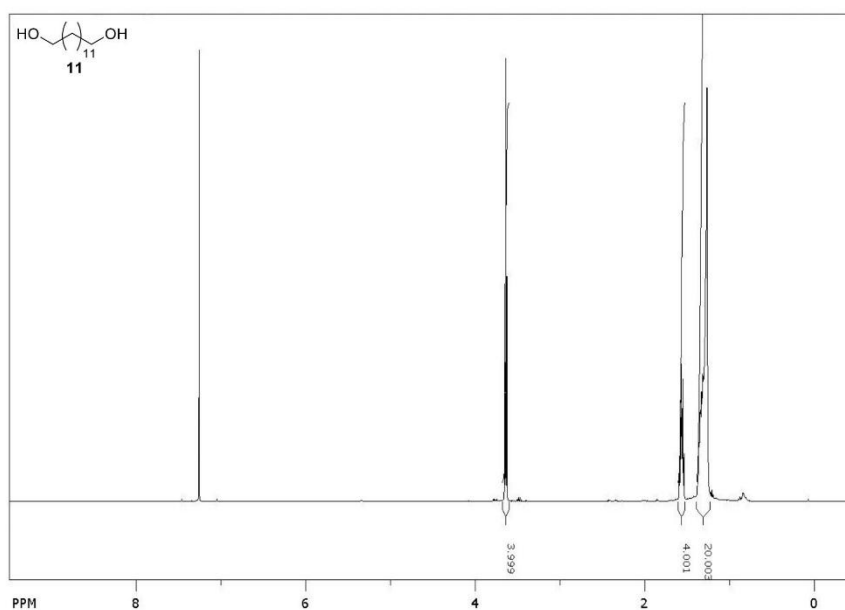


Figure 3 1,13-Tridecanediol (**11**), ^1H NMR (500 MHz, CDCl_3).

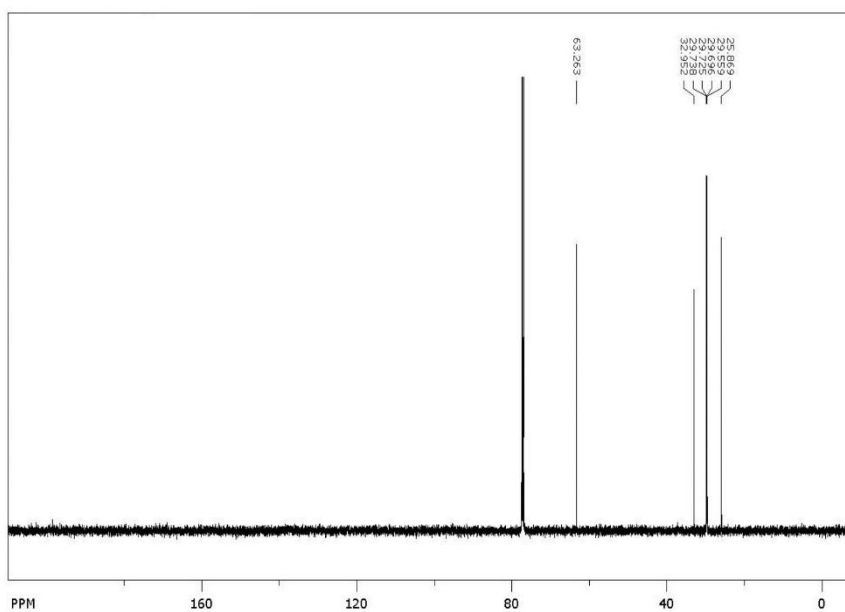


Figure 4 1,13-Tridecanediol (**11**), ^{13}C NMR (125 MHz, CDCl_3).

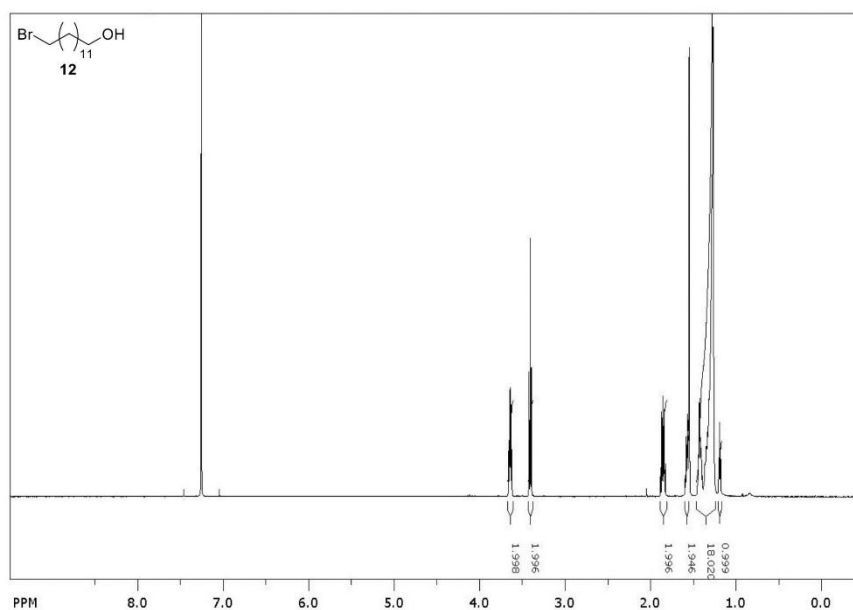


Figure 5 13-Bromotridecan-1-ol (**12**), ¹H NMR (500 MHz, CDCl₃).

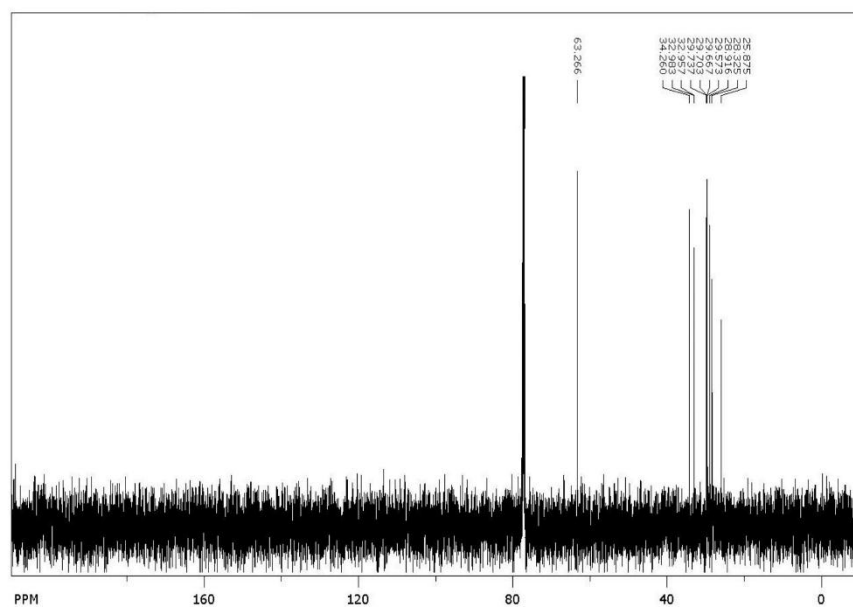


Figure 6 13-Bromotridecan-1-ol (**12**), ¹³C NMR (125 MHz, CDCl₃).

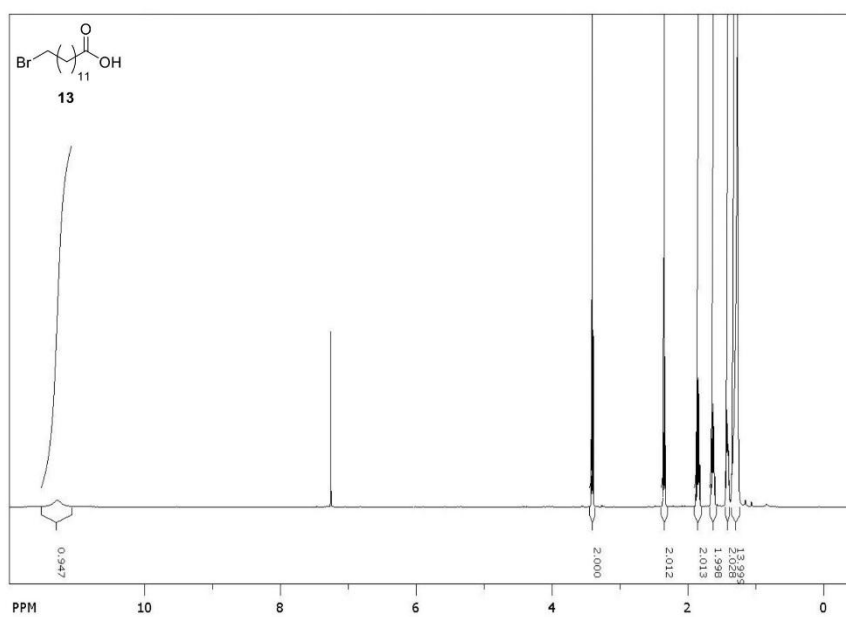


Figure 7 13-Bromotridecanoic acid (**13**), ¹H NMR (500 MHz, CDCl₃).

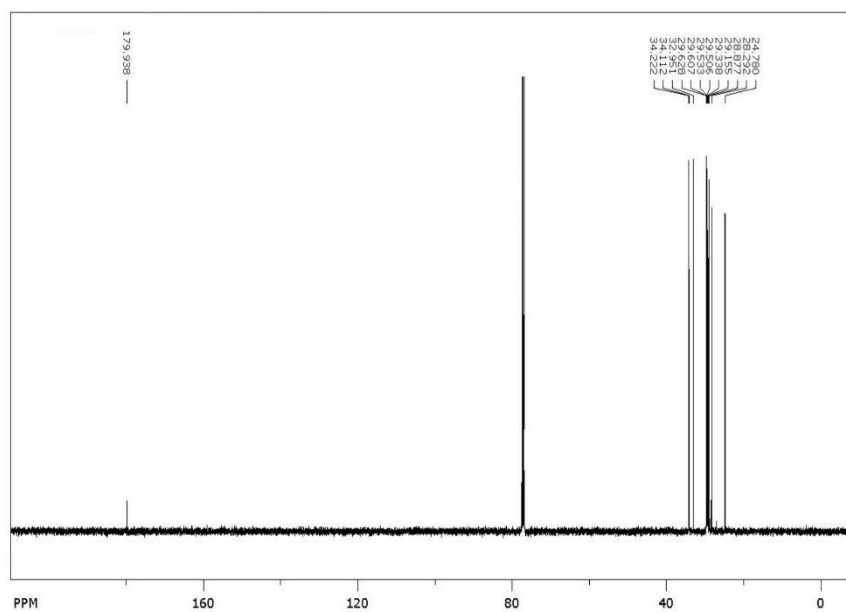


Figure 8 13-Bromotridecanoic acid (**13**), ¹³C NMR (125 MHz, CDCl₃).

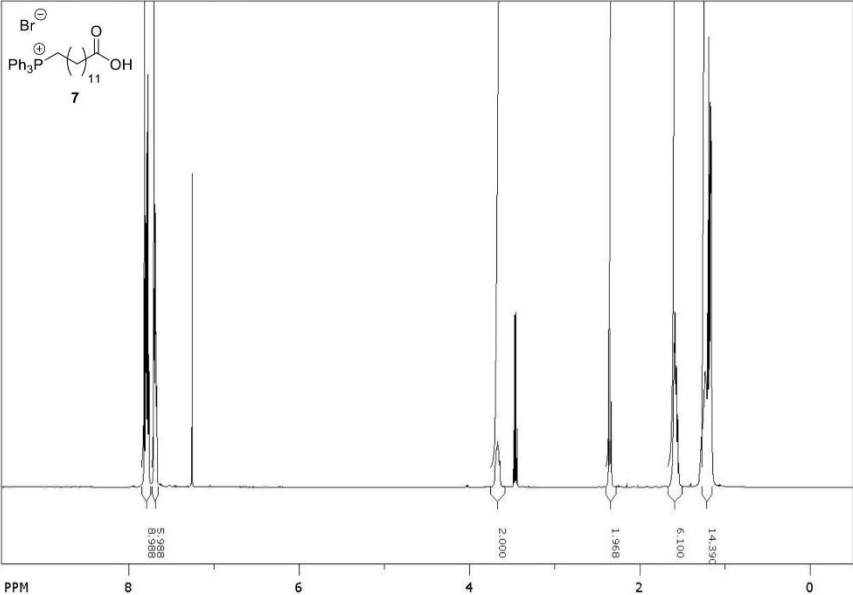


Figure 9 12-Carboxydodecyltriphenylphosphonium bromide (7), ^1H NMR (500 MHz, CDCl_3).

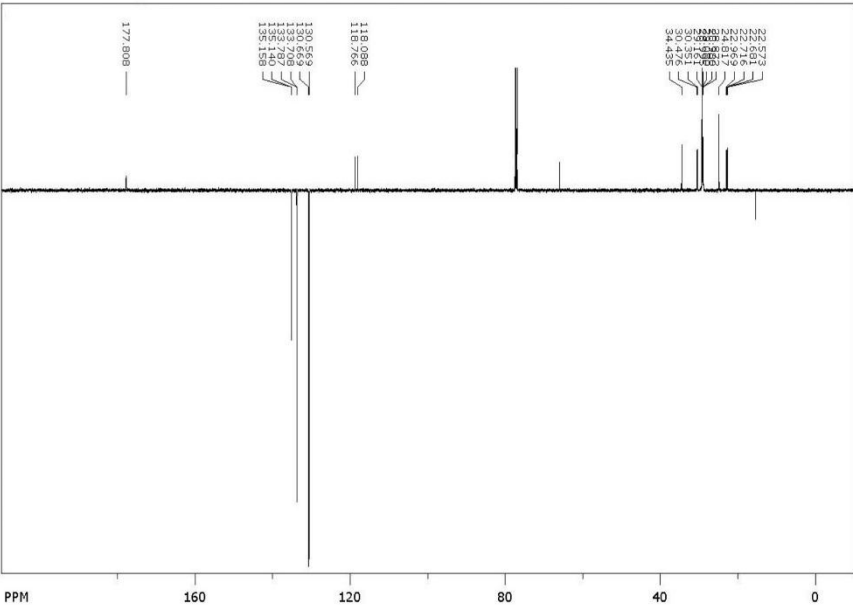


Figure 10 12-Carboxydodecyltriphenylphosphonium bromide (**7**), ^{13}C NMR (125 MHz, CDCl_3).

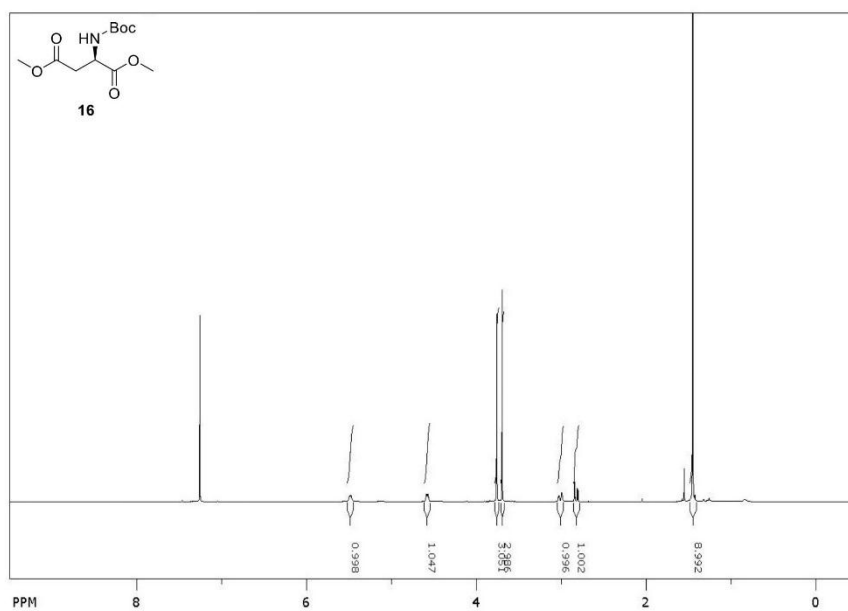


Figure 11 d-N-Boc-Asp(OMe)-OMe (**16**), ¹H NMR (500 MHz, CDCl₃).

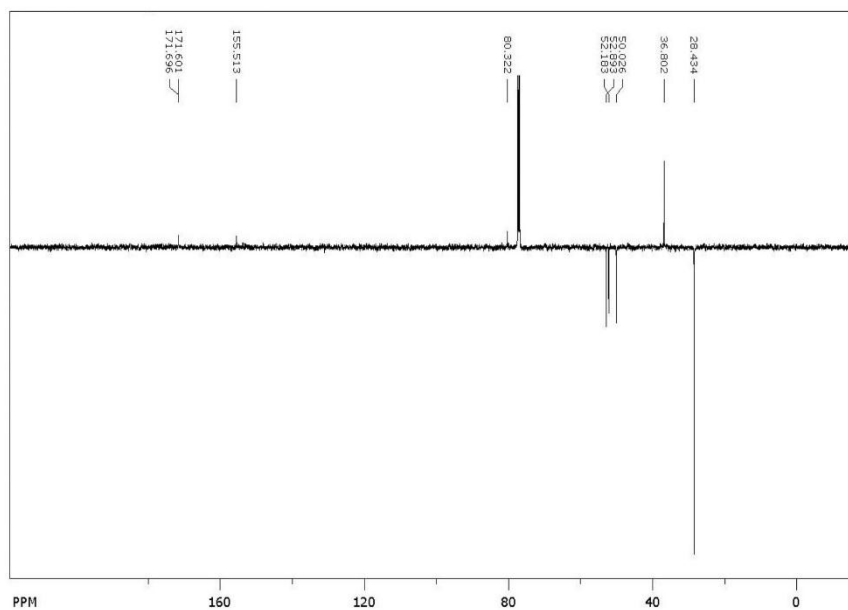


Figure 12 d-N-Boc-Asp(OMe)-OMe (**16**), ¹³C NMR (125 MHz, CDCl₃).

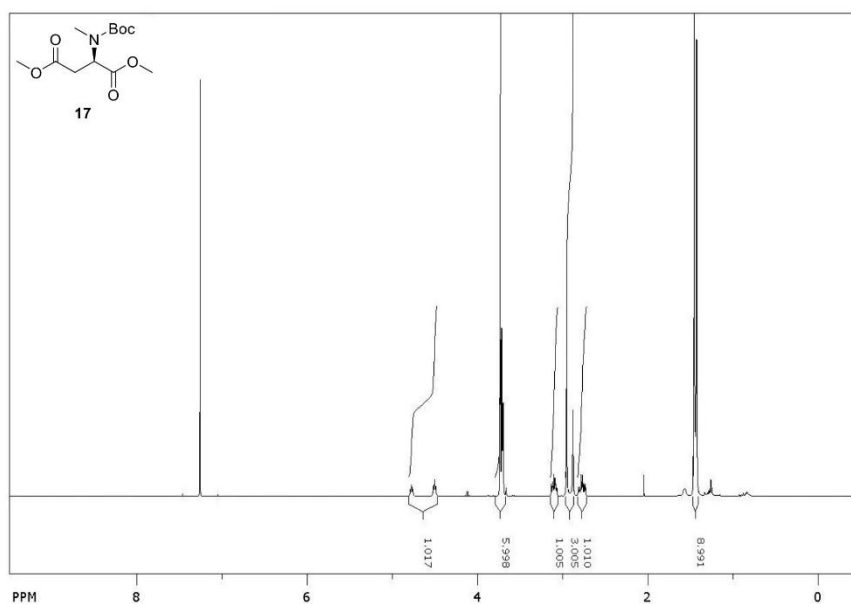


Figure 13 D-N-Me-N-Boc-Asp(OMe)-OMe (**17**), ¹H NMR (500 MHz, CDCl₃).

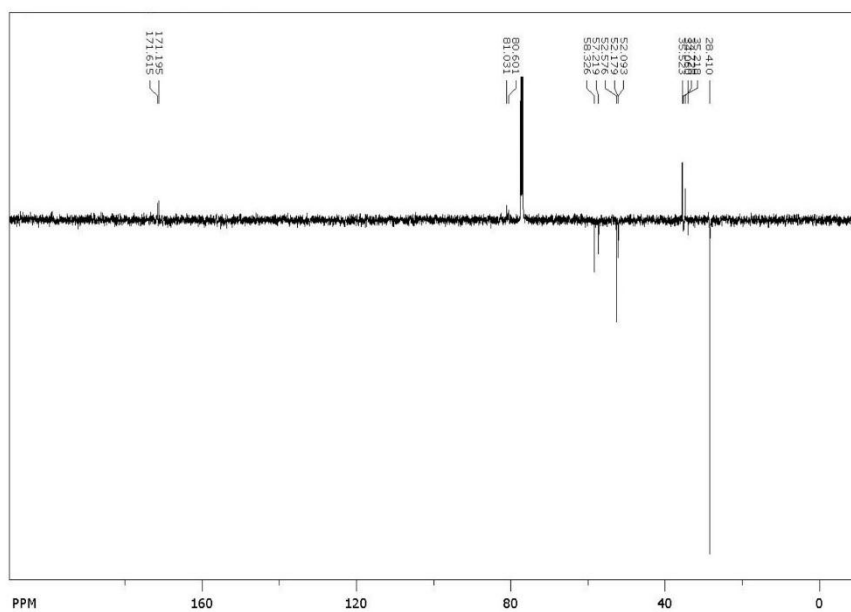


Figure 14 D-N-Me-N-Boc-Asp(OMe)-OMe (**17**), ¹³C NMR (125 MHz, CDCl₃).

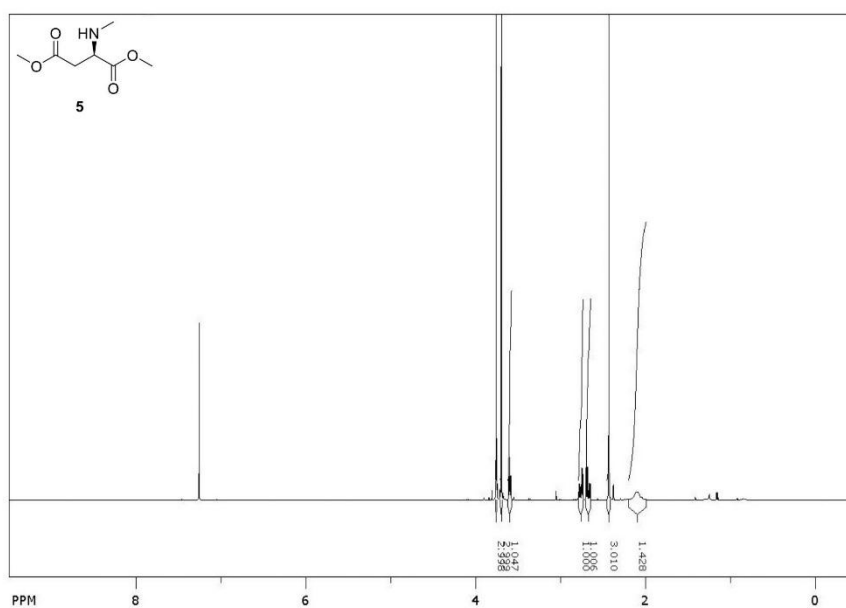


Figure 15 D-N-Me-Asp(OMe)-OMe (**5**), ¹H NMR (500 MHz, CDCl₃).

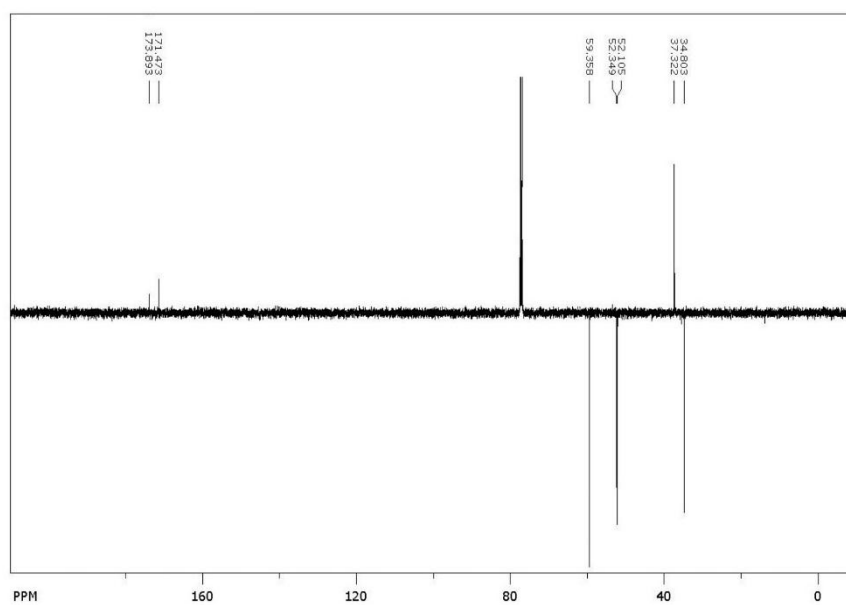
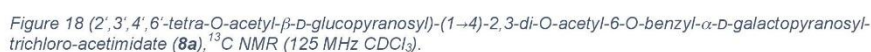
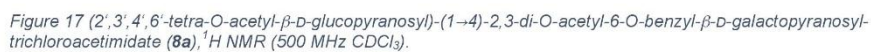


Figure 16 D-N-Me-Asp(OMe)-OMe (**5**), ¹³C NMR (125 MHz, CDCl₃).



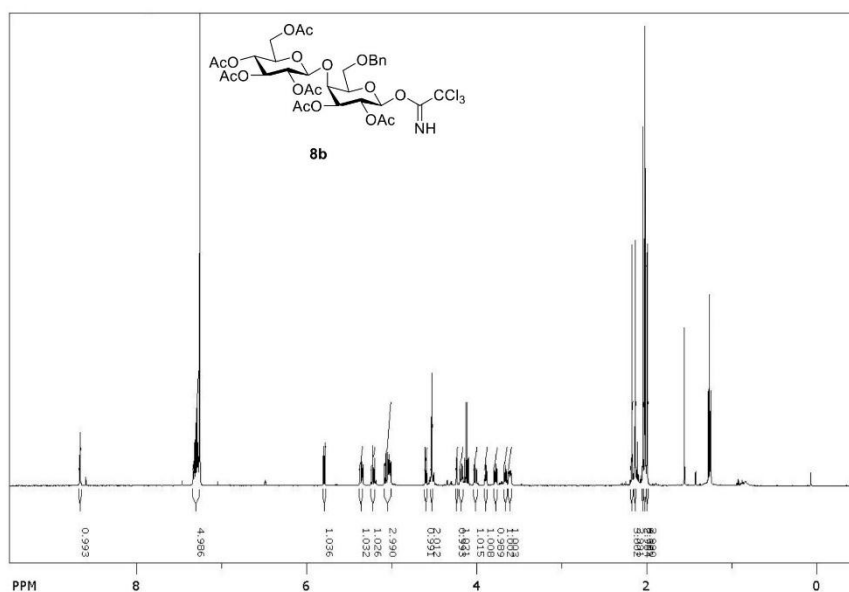


Figure 19 (2',3',4',6'-tetra-O-acetyl-β-D-glucopyranosyl)-(1 → 4)-2,3-di-O-acetyl-6-O-benzyl-β-D-galactopyranosyl-trichloro-acetimidate (**8b**), ¹H NMR (500 MHz CDCl₃).

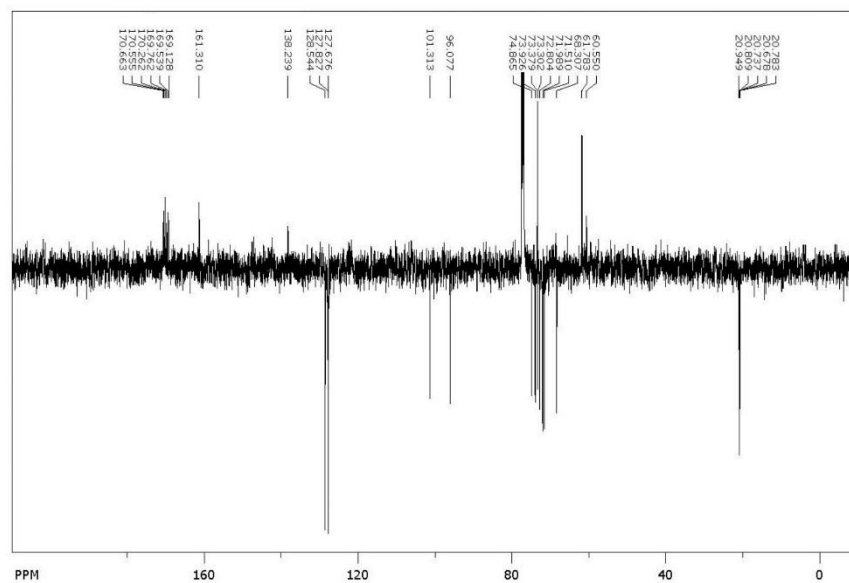
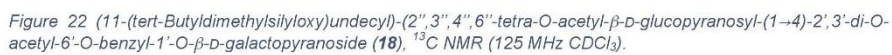
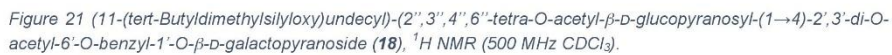


Figure 20 (2',3',4',6'-tetra-O-acetyl-β-D-glucopyranosyl)-(1 → 4)-2,3-di-O-acetyl-6-O-benzyl-β-D-galactopyranosyl-trichloro-acetimidate (**8b**), ¹³C NMR (125 MHz CDCl₃).





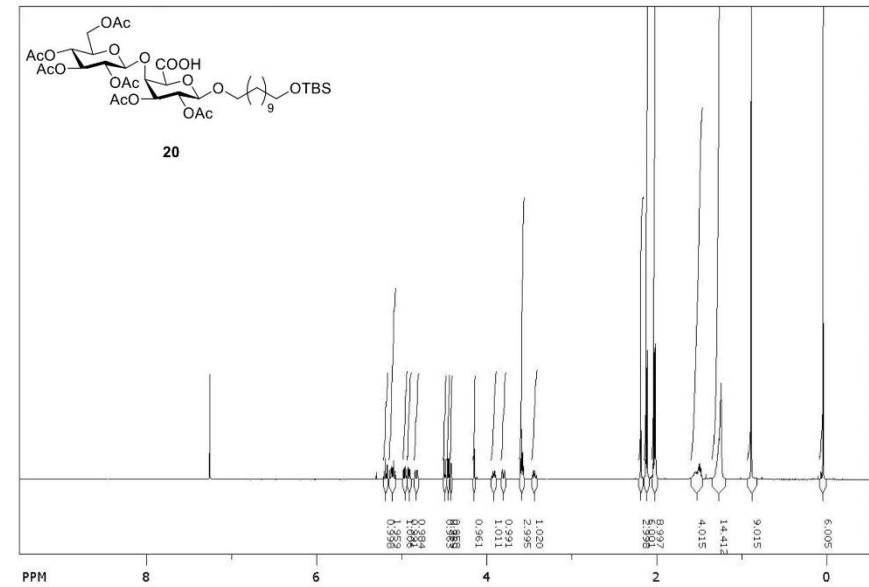


Figure 25 (11-(tert-Butyldimethylsilyloxy)undecyl)-(2',3'',4'',6''-tetra-O-acetyl- β -D-glucopyranosyl-(1 \rightarrow 4)-2',3'-di-O-acetyl-1'-O- β -D-galactopyranosiduronic acid (**20**), ^1H NMR (500 MHz CDCl_3).

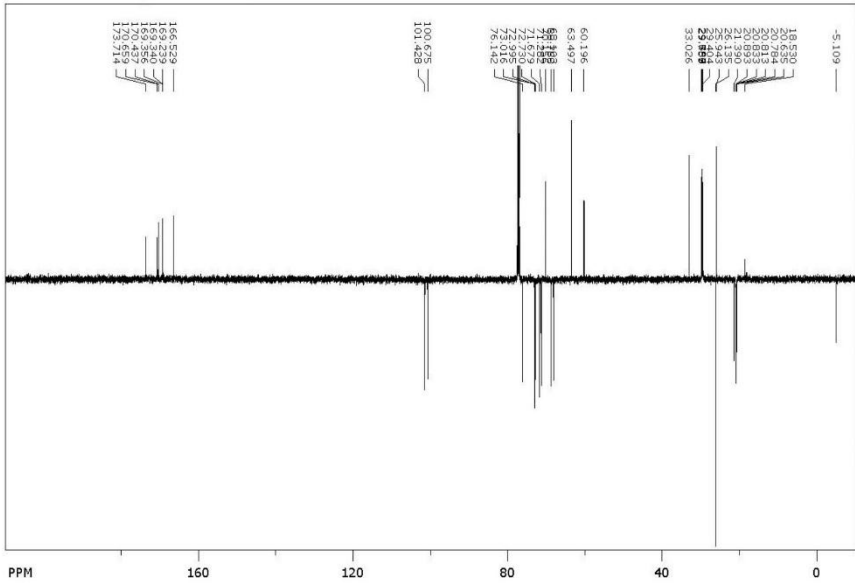


Figure 26 (11-(*tert*-Butyldimethylsilyloxy)undecyl)-(2',3'',4'',6''-tetra-*O*-acetyl- β -D-glucopyranosyl-(1 \rightarrow 4)-2',3'-di-*O*-acetyl-1'-*O*- β -D-galactopyranosiduronic acid (**20**), ^{13}C NMR (125 MHz CDCl_3).

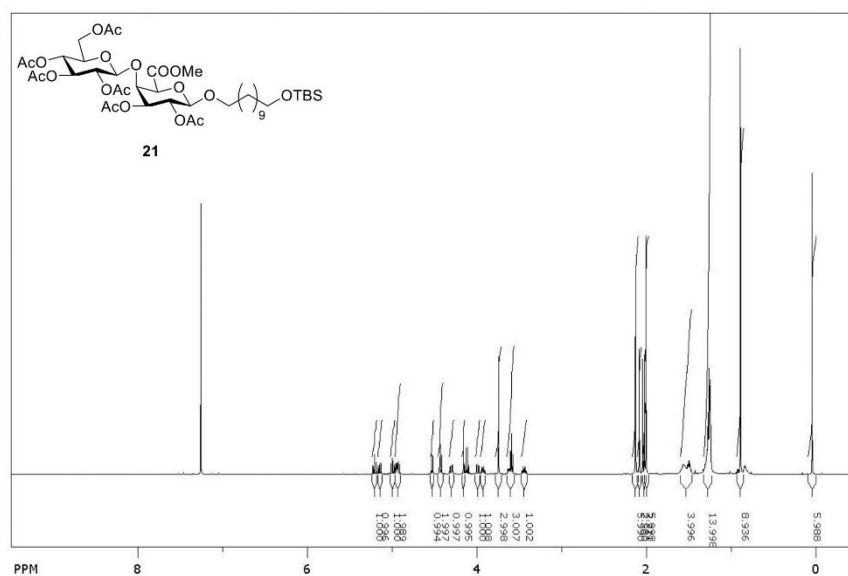


Figure 27 Methyl-((11-(tert-butyl dimethylsilyloxy)undecyl)-2'',3'',4'',6''-tetra-O-acetyl-β-D-glucopyranosyl-(1→4)-2',3'-di-O-acetyl-1'-O-β-D-galactopyranoside)uronate (**21**), ¹H NMR (500 NMR CDCl₃).

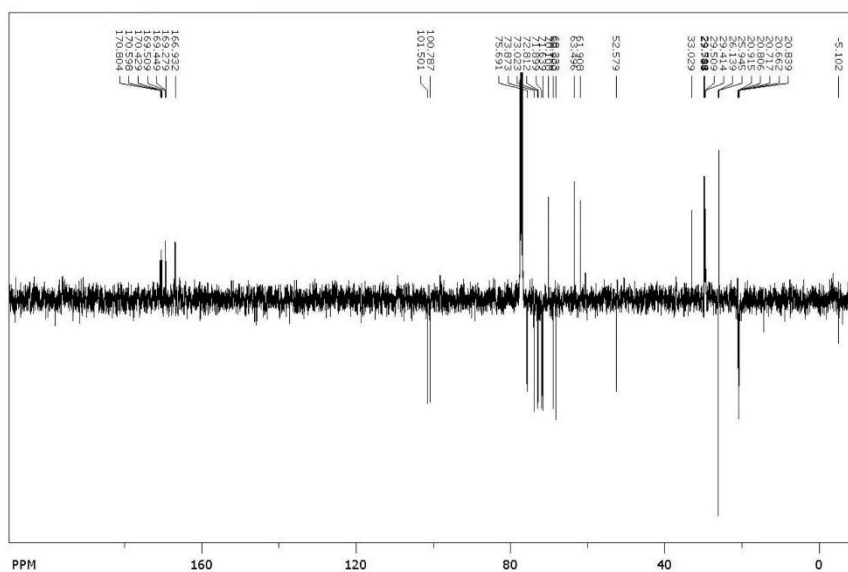


Figure 28 Methyl-((11-(tert-butyl dimethylsilyloxy)undecyl)-2'',3'',4'',6''-tetra-O-acetyl-β-D-glucopyranosyl-(1→4)-2',3'-di-O-acetyl-1'-O-β-D-galactopyranoside)uronate (**21**), ¹³C NMR (125 NMR CDCl₃).

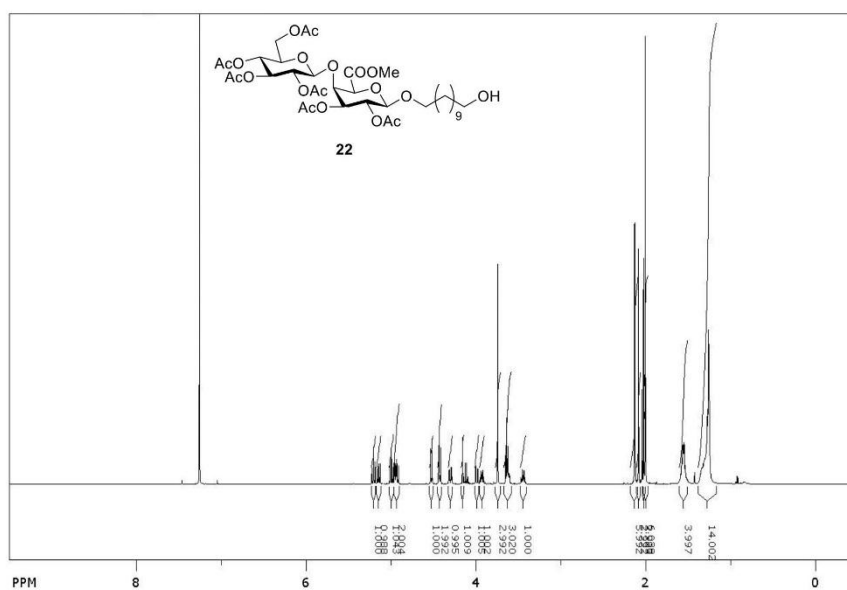


Figure 29 Methyl-((11-hydroxyundecyl)-2'',3'',4'',6''-tetra-*O*-acetyl- β -D-glucopyranosyl-(1 \rightarrow 4)-2',3'-di-*O*-acetyl-1'-*O*- β -D-galactopyranoside)uronate (22), ¹H NMR (500 NMR CDCl₃).

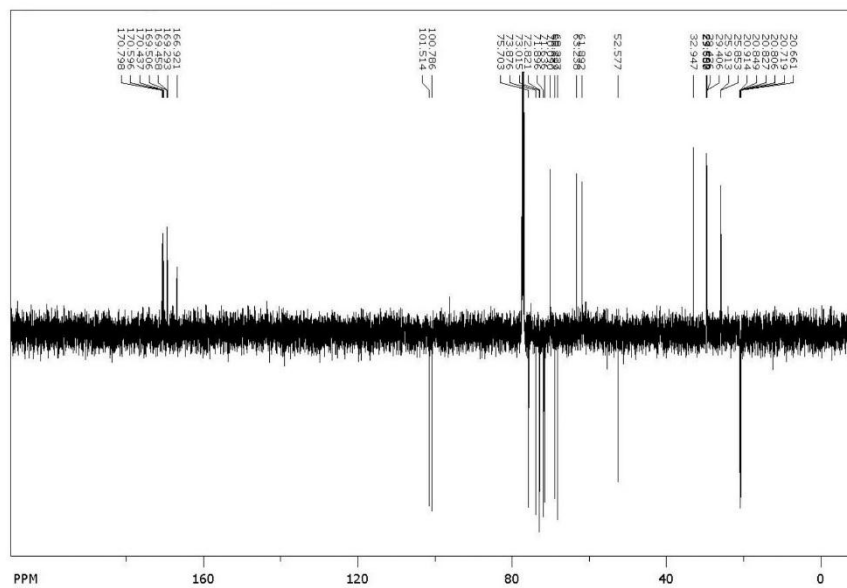


Figure 30 Methyl-((11-hydroxyundecyl)-2'',3'',4'',6''-tetra-*O*-acetyl- β -D-glucopyranosyl-(1 \rightarrow 4)-2',3'-di-*O*-acetyl-1'-*O*- β -D-galactopyranoside)uronate (22), ¹³C NMR (125 NMR CDCl₃).

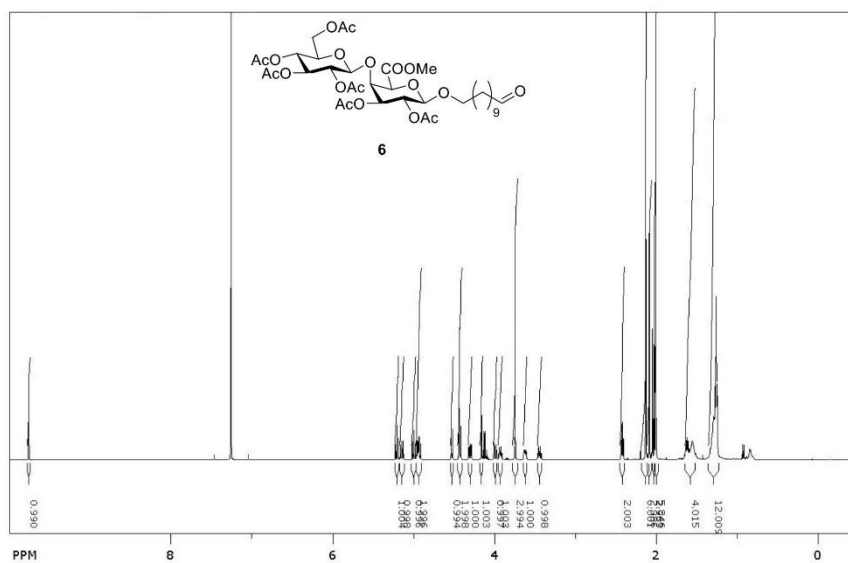


Figure 31 Methyl-((11-oxoundecyl)-2'',3'',4'',6''-tetra-O-acetyl-β-D-glucopyranosyl-(1→4)-2',3'-di-O-acetyl-1'-O-β-D-galactopyranoside)uronate (6), ¹H NMR (500 NMR CDCl₃).

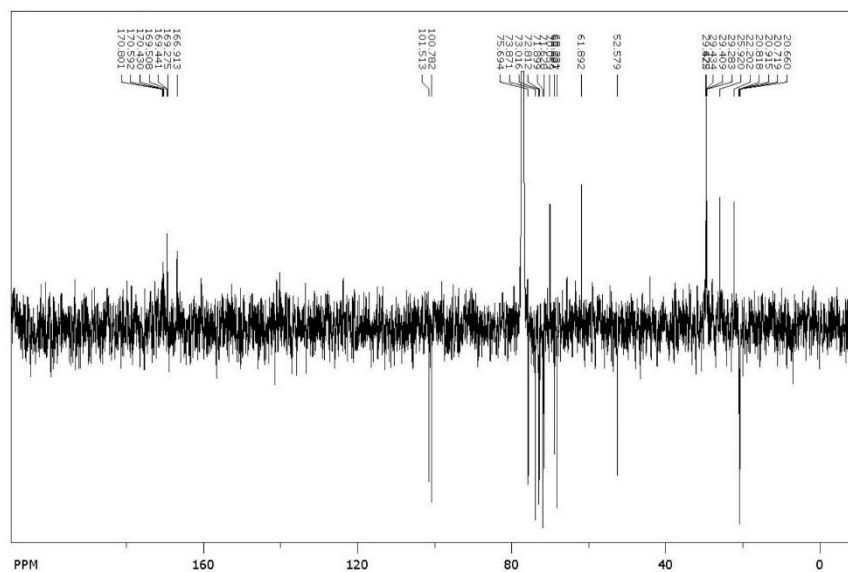


Figure 32 Methyl-((11-oxoundecyl)-2'',3'',4'',6''-tetra-O-acetyl-β-D-glucopyranosyl-(1→4)-2',3'-di-O-acetyl-1'-O-β-D-galactopyranoside)uronate (6), ¹³C NMR (125 NMR CDCl₃).

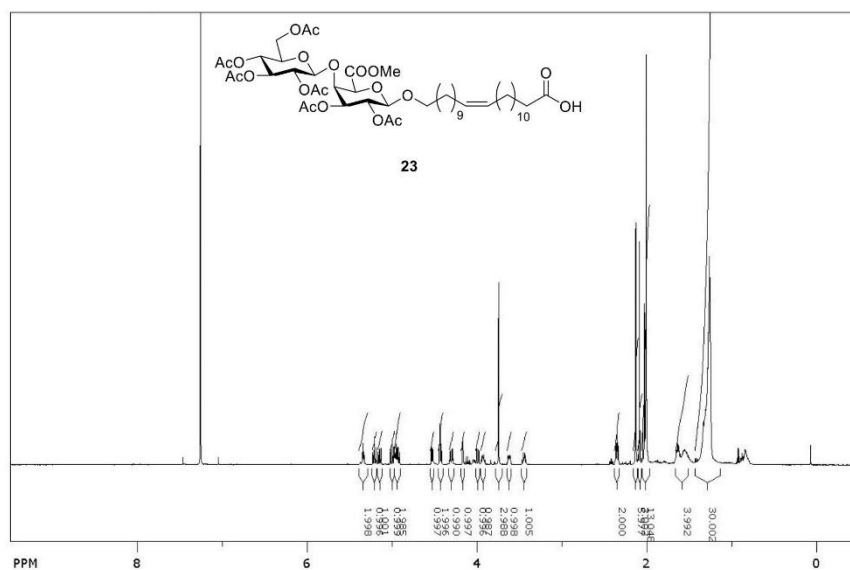


Figure 33 Methyl-(Z)-23-carboxy-tricos-11-enyl-(2'',3'',4'',6'')-tetra-O-acetyl- β -D-glycopyranosyl)-(1 \rightarrow 4)-2',3'-di-O-acetyl-1'-O- β -D-galactopyranosideuronate (**23**), ^1H NMR (500 MHz CDCl_3).

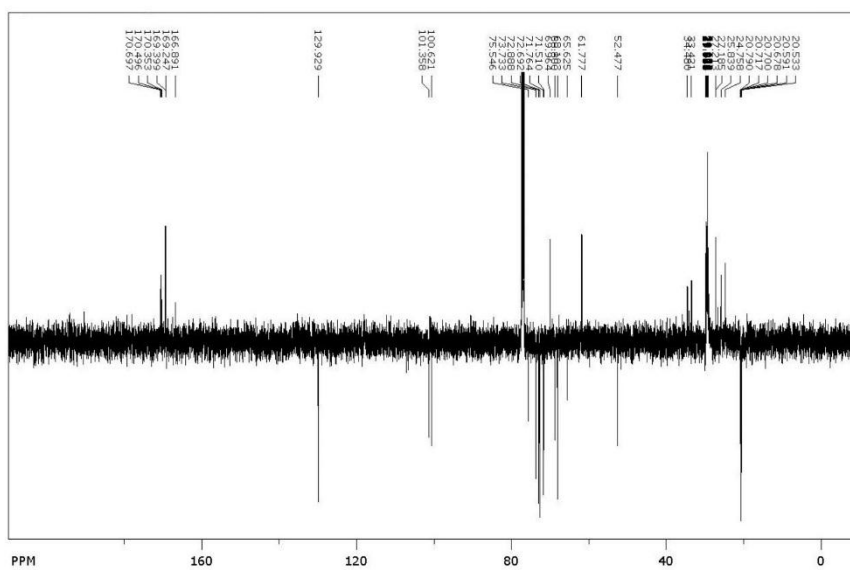


Figure 34 Methyl-(Z)-23-carboxy-tricos-11-enyl-(2'',3'',4'',6'')-tetra-O-acetyl- β -D-glycopyranosyl)-(1 \rightarrow 4)-2',3'-di-O-acetyl-1'-O- β -D-galactopyranosideuronate (**23**), ^{13}C NMR (125 MHz CDCl_3).





Liste der Publikationen

- Markus Petermichl, Sebastian Loscher, Rainer Schobert, *Total Synthesis of Aurantoside G, an N- β -Glycosylated 3-Oligoenoyltetramic Acid from Theonella swinhoe*, *Angew. Chem. Int. Ed.* **2016**, 55, 10122-10125; Markus Petermichl, Sebastian Loscher, Rainer Schobert, *Totalsynthese von Aurantosid G einer N- β -glykosylierten 3-oligoenoyltetramsäure aus Theonella swinhoe*, *Angew. Chem.* **2016**, 128, 10276-10279.
- Markus Petermichl, Rainer Schobert, *3-Acyltetramic Acids: A Decades-Long Approach to a Fascinating Natural Product Family*, *Synlett.* **2017**, 28, 654-663.
- Markus Petermichl, Rainer Schobert, *Total Synthesis of the Diglycosidic Tetramic Acid Ancorinoside A*, *Chem. Eur. J.* **2017**, 23, 14743-14746.
- Markus Petermichl, Christine Steinert, Rainer Schobert, *A Synthetic Route to the MT1-MMP Inhibitor Ancorinoside D*, *Synthesis*, **2019**, 51, 730-738.

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